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AUSTRALASIAN ANNALS OF MEDICINE

Journal of The Royal Australasian College of Physicians

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THE LOCALIZATION AND BINDING OF SERUM PROTEINS IN THE GLOMERULI OF KIDNEY BIOPSIES IN DISSEMINATED LUPUS ERYTHEMATOSUS AND GLOMERULONEPHRITIS[1]

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SUMMARY

A report has been presented on the use of fluorescent antibody (fluorescein coupled with rabbit anti-human gamma globulin), in the localization of serum protein in kidney tissue obtained by needle biopsy.

Serum protein or fractions thereof have been localized bound to the glomerular capillary walls in kidney biopsies obtained from seven patients with glomerulonephritis associated with disseminated *lupus erythematosus* or related diseases, from one patient with fatal progressive glomerulonephritis and to a lesser degree from one patient with diabetic nephropathy. No such localization could be demonstrated in the glomeruli of seven normal kidneys and in ten other cases of kidney disease.

It is suggested that this binding of serum protein represents an antigen-antibody reaction. Alternatively it may be due to a secondary adsorption of plasma by the damaged capillary wall, or a combination of these processes.

Since the demonstration by Masugi (1933) that glomerulonephritis can be induced in experimental animals by the injection of an antiserum prepared against their own kidney tissues, attempts have been made to show that glomerulonephritis in humans is the result of an antigen-antibody reaction occurring in the glomerulus.

The identification and localization of antigenic substances in tissues has been achieved by fluorescent antibody staining techniques (Coons and Kaplan, 1950). This technique has been applied to experimental glomerulonephritis in rats, and localization of antibody globulin in the affected glomeruli has been demonstrated (Mellors *et alii*, 1955). Mellors and Ortega (1956) similarly demonstrated the localization of gamma globulin in the glomerulus in fatal cases of acute glomerulonephritis, lipid nephrosis and other conditions by utilizing a fluorescein-coupled rabbit anti-human gamma globulin as a staining reagent. They suggested that this was further evidence that certain types of glomerulonephritis were due to interaction of the patient's immune mechanisms against his own kidney tissues, even though the original antigenic stimulus might be of exogenous origin.

Because there is minimal denaturation of the proteins, the fresh tissue obtained in the course of a diagnostic kidney biopsy is particularly suited to fluorescent antibody study. To our knowledge, fluorescent antibody techniques have not been previously applied to kidney biopsies The present paper reports our experiences with this technique in patients with disseminated *lupus erythematosus* (D.L.E.) and other forms of glomerulonephritis.

MATERIALS AND METHODS

Clinical Material and Kidney Biopsy

Kidney tissue was obtained from 24 patients. Nine patients had D.L.E. confirmed by positive results from L.E. cell tests, while one patient had lupoid hepatitis—chronic hepatitis with L.E. cells (Mackay *et alii*, 1956). This case had been reported in full (Taft *et alii*, 1958). The clinical features and diagnoses of these and the remaining 14 patients are set out in Table I.

[1] Received on August 27, 1957.

[2] Working with the aid of a grant from the National Health and Medical Research Council of Australia.

TABLE I

Case No.	Clinical Features	Urine	Glomerular Histology	Glomerular Fluorescence
1	Female aged 56 years. Illness 20 years, with fever, arthropathy, purpura, dyspnœa, œdema L.E. cell test positive	Protein + Red cells ++ Pus cells Granular and hyaline casts	Severe basement membrane thickening and focal necrosis, capillary occlusion, capsular adhesions, capsular protein Appearance characteristic of lupus glomerulonephritis	Strongly positive
2	Female aged 34 years. Illness six years, with fever, rash, myalgia, Raynaud's phenomenon, pleurisy, œdema and hypertension L.E. cell test positive Partial remission with cortisone Death due to renal failure	Protein +++ Red cells ++ Pus cells Granular and hyaline casts	Severe basement membrane thickening, capillary occlusion, capsular adhesions, capsular protein Appearance characteristic of lupus glomerulonephritis	Strongly positive. Confirmed *post mortem*
3	Female aged 25 years. Illness two years, with fever, rash, arthropathy, myalgia, pleurisy, nephrotic syndrome. L.E. cell test positive Remission with cortisone	Protein + Granular and hyaline casts	Moderate basement membrane thickening, capsular adhesions and some epithelial proliferation Appearance consistent with lupus glomerulonephritis	Positive
4	Female aged 38 years. Illness five years, with fever, rash, arthropathy, myalgia, hæmolytic anæmia L.E. cell test positive Remission with cortisone and splenectomy	Normal	Glomeruli normal (Interstitial fibrosis) (Tubular casts)	Negative
5	Female aged 39 years. Illness four years, with fever, arthropathy, pericarditis, pleurisy thrombophlebitis L.E. cell test positive Remission with cortisone	Normal	Normal	Negative
6	Female aged 22 years. Illness six months, with fever, arthropathy, jaundice L.E. cell test positive	Normal	Normal	Negative
7	Female aged 23 years. Illness one year, with fever, rash, arthropathy, purpura L.E. cell test positive Remission with cortisone	Normal	Occasional glomeruli show moderately severe basement membrane thickening, capillary occlusion, capsular adhesions Appearance consistent with lupus glomerulonephritis	Positive
8	Female aged 29 years. Illness 11 years, with fever, arthropathy, nephrotic syndrome L.E. cell test positive. Partial response to very large doses of cortisone	Protein ++ Occasional red cell, pus cell	Considerable basement membrane thickening with some focal necrosis, capillary occlusion, capsular adhesions, capsular protein Appearance characteristic of lupus glomerulonephritis	Strongly positive
9	Female aged 20 years. Fever, arthropathy, rash L.E. cell test positive Remission with cortisone	Normal	Normal	Negative
10	Female aged 23 years. Illness three years, with jaundice, proteinuria, ascites and diarrhœa L.E. cell test positive Slight remission with cortisone	Protein ++ Occasional red cell, pus cell, hyaline casts	Moderately severe basement membrane thickening and endothelial hyperplasia Appearance consistent with lupus glomerulonephritis	Strongly positive
11	Male aged 54 years. Past history of arthritis, severe œdema 21 months L.E. cell test negative Considerable remission with cortisone	Protein ++++ Granular casts	Severe basement membrane thickening, capillary occlusion, occasional fine capsular adhesion Appearance consistent with lupus glomerulonephritis	Strongly positive on two separate occasions
12	Male aged 40 years. Follicular hyperkeratosis of skin. Severe œdema for seven months L.E. cell test negative Poor response to cortisone	Protein ++++ Occasional red cell and pus cell	Normal (Tubular dilatation)	Negative
13	Male aged 48 years. Nephrotic syndrome of six weeks' duration L.E. cell test negative Remission with cortisone	Protein ++ Occasional cast	Increased cellularity, capsular adhesions, capillary occlusion with slight basement membrane thickening Appearance that of a progressive subacute glomerulonephritis	Negative
14	Male aged 48 years. Fatigue, dyspnœa, œdema and asthma over one year L.E. cell test negative Spontaneous remission	Protein ++ Occasional red cell	Capsular adhesions, capsular protein, capillary occlusion with slight basement membrane thickening Appearance that of a progressive subacute glomerulonephritis	Negative
15	Female aged 43 years. *Diabetes mellitus* 18 years. Hypertension, œdema, retinopathy	Protein ++ Occasional granular and hyaline cast	Slight to moderate basement membrane thickening consistent with early diabetic nephropathy	Positive

TABLE I—*Continued.*

Case No.	Clinical Features	Urine	Glomerular Histology	Glomerular Fluorescence
16	Male aged 54 years. Nutritional hepatitis developing oliguria, œdema and uræmia Antistreptolysin titre : 1 : 400 Serum gamma globulin : 2·5 grammes *per centum*	Protein ++ Red cells +++ Occasional pus cells	Hypercellularity and polymorphonuclear infiltration Appearance characteristic of acute glomerulonephritis	Negative
17	Male aged 43 years. Bronchial asthma, pneumonia, developing œdema and oliguria Antistreptolysin titre : 1 : 400 Serum gamma globulin : 2·3 grammes *per centum*	Protein ++ Red cells +++ Pus cells Numerous granular and cellular casts	Hypercellularity and polymorphonuclear infiltration Appearance characteristic of acute glomerulonephritis	Negative
18	Male aged 41 years. Hæmatemeses, hæmoptyses, anæmia and uræmia developing over two months. Death from pulmonary œdema Antistreptolysin titre : 1 : 50 Serum gamma globulin : 1·1 grammes *per centum*	Protein ++ Red cells Pus cells. Casts	Hypercellularity, capillary occlusion and disorganization, capsular adhesions Appearance that of a severe progressive glomerulonephritis	Positive
19	Male aged 25 years. Acute glomerulonephritis when aged 11 years. Dyspnœa, hæmoptyses, œdema over one year	Protein ++ Occasional red cell, pus cell, granular casts	Epithelial and endothelial proliferation, capsular adhesions, capillary disorganization and progressive fibrosis Appearance that of a severe progressive glomerulonephritis entering the chronic stage	Negative
20	Male aged 45 years. Oliguria, œdema, four days. Hypertension, persistent uræmia	Protein ++ Occasional granular and hyaline casts	Glomerular hypercellularity and fibrosis, with basement membrane thickening Appearance that of a chronic membranous glomerulonephritis	Negative
21	Female aged 53 years. Progressive essential hypertension five years. Papillœdema, encephalopathy, anæmia	Protein ++ Red cells ++ Occasional pus cells and casts	Glomerular fibrosis (Severe hyaline thickening, tortuosity and occlusion of juxtaglomerular arterioles.) Appearance that of an advanced progressive benign hypertension	Negative
22	Female aged 28 years. Idiopathic epilepsy, developing severe hypertension, papillœdema	Protein ++ Occasional red cell and pus cell	Early tuft necrosis (Early necrosis in juxtaglomerular arteriole) Appearance that of the early changes seen in malignant hypertension	Negative
23	Male aged 46 years. Acute oliguria progressing to anuria and uræmia over one week	Protein +++ Pus cells +++ Granular casts ++	Some leucocytic infiltration (Tubular casts) (Interstitial inflammation) Appearance that of a severe interstitial nephritis	Negative
24	Female aged 41 years. Rheumatoid arthritis 30 years L.E. cell test negative	Normal	Normal	Negative

Percutaneous aspiration biopsy of the kidney with a Franseen needle (Joske, 1954), using a posterior approach, was performed on 22 patients. The only complication following the biopsies was the occurrence of a perinephric abscess in one patient on a high dose of cortisone. The abscess required drainage. Kidney tissue was also obtained in two fatal cases by needle aspiration immediately *post mortem*. Normal control kidney tissue was obtained at necropsy from three patients who had died of extraneous diseases.

Renal cortex was identified by low power microscopy of the biopsy specimen, and a fragment was immediately embedded in liquid 10% gelatine at 37° C, cooled to 4° C until set, and from a small block sections 10–15 μ in thickness were cut with a freezing microtome. The sections were momentarily floated out in water, transferred to egg albuminized slides and dried *in vacuo*. The remainder of the biopsy was processed for routine histological study, including periodic acid-Schiff and Mallory's phosphotungstic acid hæmatoxylin stains.

Preparation of Fluorescent Rabbit Anti-Human Gamma Globulin

Two millilitres of electrophoretically homogeneous human serum gamma globulin (Commonwealth Serum Laboratories ; 160 milligrammes of protein per millilitre) were emulsified with 0·25 millilitres of sterile adjuvants " Arlacel A " and 1·5 millilitres of " Bayol F ". The emulsion was injected subcutaneously into the thighs of an adult male rabbit, which was bled from the marginal ear vein four weeks after

injection. The globulin fraction was precipitated from the rabbit antiserum with 50% ammonium sulphate solution and coupled with fluorescein isocyanate as described by Coons and Kaplan (1950). The fluorescent rabbit anti-human

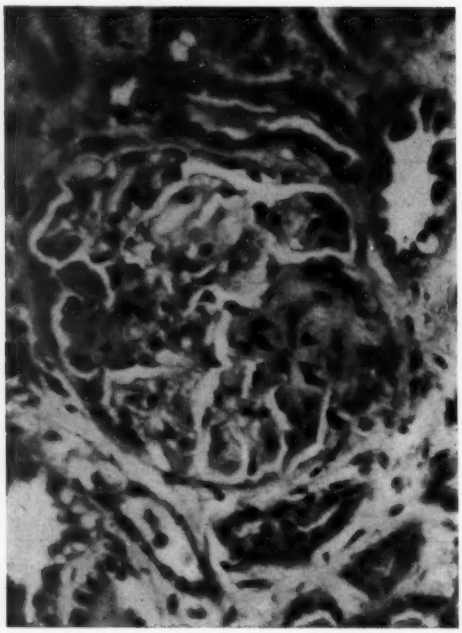

FIGURE I

Glomerulus from kidney biopsy in Case No. 1 with severe lupus glomerulonephritis. There is a nodular thickening of the basement membrane with capillary occlusion and capsular adhesions. (H. & E., ×400)

gamma globulin was dialyzed against isotonic phosphate buffered saline (pH 7·3) and stored at —17° C, after the addition of merthiolate to a dilution of 1 : 10,000. To remove unbound fluorescein derivatives, the fluorescent reagent was absorbed with acetone-extracted mouse liver immediately before use.

Staining Technique and Photography

The method of staining was similar to that described by Mellors and Ortega (1956). The sections were washed in isotonic phosphate buffered saline at pH 7·0, stained with the fluorescent antibody for ten minutes and rewashed. The sections were then mounted in neutral phosphate buffered glycerol. The microscope and illuminating system used in these studies has been described by Matthaei (1950). Photomicrographs were taken with Kodak "Tri X" 35 millimetres film immediately after staining, as fluorescence disappeared after 24 hours. Assessment of fluorescence of different structures was made visually by comparison with surrounding tissues.

Specificity of the Stain

Antisera were obtained from five rabbits and each titrated against human serum albumin and gamma globulin using the ring precipitin test. Both of these serum fractions reacted to titres of between 1 : 1000 and 1 : 3000, so that it cannot be stated that gamma globulin is the sole antigen.

The kidney tissue obtained at necropsy in Case 10 provided sufficient tissue to demonstrate

FIGURE II

Glomerulus from kidney biopsy in Case No. 1 stained with fluorescent antibody (serum from a rabbit immunized with human γ globulin, and conjugated with fluorescein isocyanate). There is a nodular fluorescence of the capillary wall. ×400.

that the staining was due to the immune rabbit serum and not to any type of non-specific effect. Specific inhibition of staining by prior incubation of the tissue sections with uncoupled rabbit

antiserum was obtained (Figure VI), while prior incubation with normal rabbit serum did not inhibit the reaction (Figure V).

These reactions and the failure to stain normal control kidney tissue (Figure III) lead us to believe that positive staining with our reagent specifically detects the presence of serum protein or fractions thereof.

RESULTS

In each case the clinical features, results of chemical and microscopic analysis of urine just prior to biopsy, kidney histology and glomerular fluorescence are shown in Table I. The occurrence of glomerular fluorescence is correlated with the diagnosis and the presence of proteinuria in Table II.

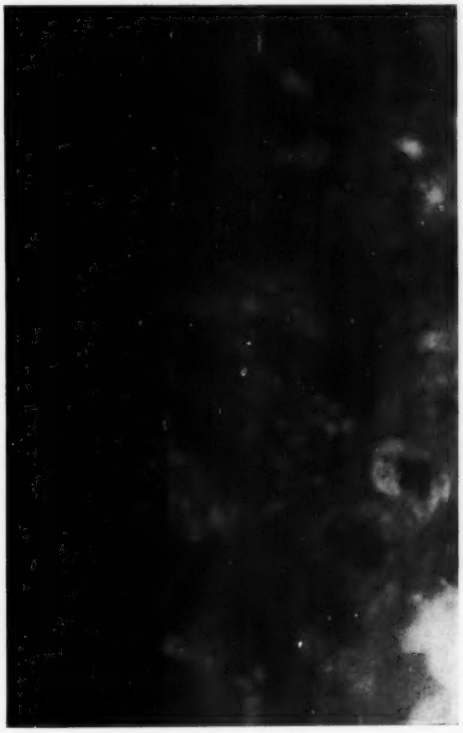

FIGURE III

Normal kidney obtained *post mortem* stained with fluorescent antibody. There is only faint autofluorescence of the nuclei. × 400.

Green fluorescence was observed in the glomeruli in Cases 1, 2, 3, 7, 8, 10, 11, 15 and 18. This fluorescence was limited to the capillary loops, and often more clearly to the walls of the loops (Figures V and VIII). In some areas nodular thickening of the basement membrane was associated with a nodular distribution of the fluorescence (Figures I, II, IX, X), while in others diffuse thickening of the basement membrane corresponded to diffuse fluorescence of the capillary wall (Figures IV, V, VII, VIII). Fluorescence occasionally occurred lining the parietal wall of Bowman's capsule, but only in cases where the tufts also fluoresced (Figure II). The fluorescence did not correspond in distribution to that of protein in the capsular space, as judged by the phosphotungstic acid hæmatoxylin-stained paraffin-embedded sections. Hyaline capillary thrombi were not seen in the paraffin sections and could not account for the fluorescence which occurred. In some sections normal or faintly fluorescing glomeruli were present alongside fluorescent glomeruli (Figure XIII). Fluorescence did not occur in glomerular areas occupied by proliferating or scar tissue, tubular epithelium, arterioles, interstitial capillaries or venules.

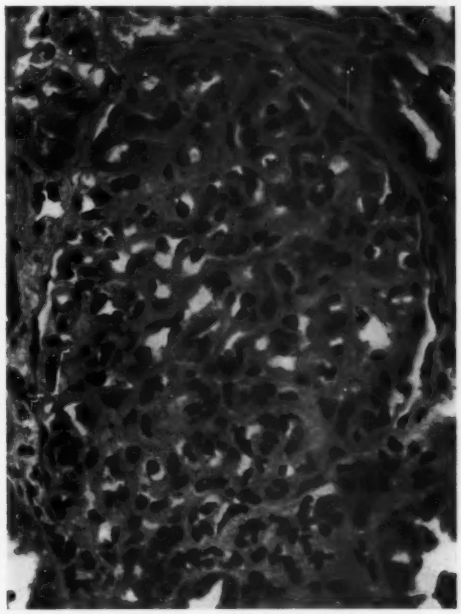

FIGURE IV

Glomerulus from kidney biopsy in Case No. 10 with lupoid hepatitis and glomerulonephritis, showing diffuse basement membrane thickening and endothelial hyperplasia. H. & E., ×400.

Five of the nine patients with D.L.E. (Cases 1, 2, 3, 7, 8) showed fluorescence located in the glomerular capillaries (Figures II, X). Despite varying degrees of clinical remission following the use of cortisone, all five had active lupus glomerulonephritis. Urinary abnormality was

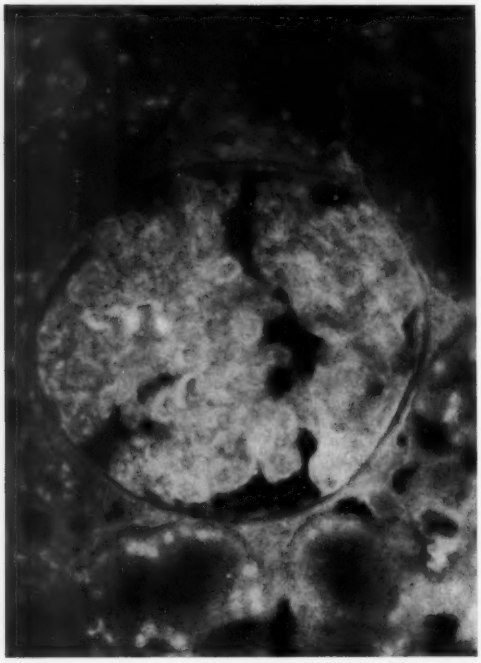

FIGURE V

Glomerulus from kidney in Case No. 10 incubated with normal rabbit serum and then stained with fluorescent antibody, showing diffuse fluorescence of capillary wall. ×350.

absent in only one case (7). The four patients (Cases 4, 5, 6, 9) who did not show glomerular fluorescence all had histologically normal glomeruli; one patient (Case 5) showed tubular and interstitial changes unassociated with any urinary abnormality. The patient (Case 10) who had lupoid hepatitis with associated glomerulonephritis also showed pronounced fluorescence of the capillary walls of the glomeruli (Figure V).

A group of five patients with the nephrotic syndrome showed varying histological changes in the kidney. In two cases glomerular fluorescence was obtained. One patient (Case 11) had a past history of arthralgia, but the result of an L.E. cell test was negative. A kidney biopsy showed severe membranous glomerulonephritis (Figure VII) and displayed a striking fluorescence of the capillary wall (Figure VIII). This patient subsequently had a further biopsy a year later, which fluoresced similarly to the first, despite partial remission of his disease while on cortisone. The other patient (Case 15) was diabetic for 18 years, and had developed a membranous glomerulonephritis. The glomerular capillary wall in this biopsy showed a weak fluorescence. Neither of the two patients (Cases 16, 17) with acute post-streptococcal glomerulonephritis showed glomerular fluorescence, although fluorescence was present *post mortem* in one fatal case with subacute glomerulonephritis.

Only faint autofluorescence (intrinsic fluorescence unassociated with the staining reagent)

FIGURE VI

Glomerulus from kidney in Case No. 10 incubated with unconjugated rabbit antibody and then stained with fluorescent antibody. The fluorescence of the capillary wall is now absent. The large white area is an artefact. ×350.

of the kidney tissues was observed in the controls (Figure III) and in Cases 4, 5, 6, 9, 12, 13, 14, 16, 17, and 19–24. This was mainly confined to the nuclei and elastic fibres, and its blue-grey or yellow colour (Figures III and VIII)

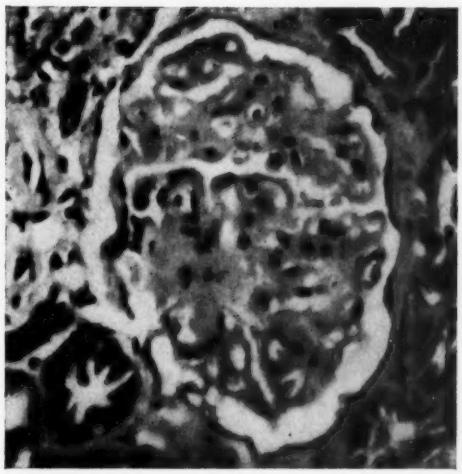

FIGURE VII

Glomerulus from kidney biopsy in Case No. 11, a male patient with arthritis and nephrotic syndrome, showing diffuse thickening of the basement membrane. H. & E., ×400.

FIGURE VIII

Glomeruli from kidney biopsy in Case No. 11 stained with fluorescent antibody. There is diffuse fluorescence of the capillary wall and autofluorescence of the elastica of a small artery in the upper left hand portion. ×150.

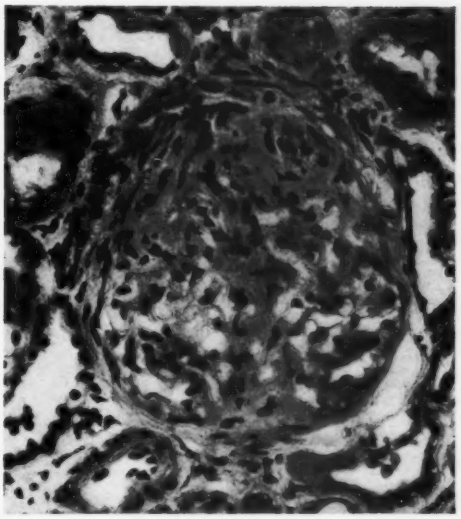

FIGURE IX

Glomerulus from kidney biopsy in Case No. 2, a female with severe lupus glomerulonephritis, showing nodular basement membrane thickening and capsular adhesions. H. & E., ×400.

FIGURE X

Glomerulus from kidney biopsy in Case No. 2 stained with fluorescent antibody. There is fluorescence of the thickened nodular capillary walls. ×400.

could be distinguished from the green fluorescence of the conjugated fluorescein. Casts in the tubules fluoresced, presumably because of incorporated serum protein or non-specific dye absorptions (Figure XI).

Other patients including those with subacute and chronic glomerulonephritis, malignant hypertension, acute interstitial nephritis and rheumatoid arthritis failed to display glomerular fluorescence in the biopsies.

TABLE II

Diagnosis	Total Number of Cases	Proteinuria	Number of Cases in which Glomerular Fluorescence Observed
D.L.E. with glomerulonephritis	5	4	5
D.L.E. without glomerulonephritis	4	—	—
Lupoid hepatitis	1	1	1
Nephrotic syndrome with arthritis	1	1	1
Rheumatoid arthritis ..	1	—	—
Diabetic nephropathy ..	1	1	1
Acute interstitial nephritis	1	1	—
Acute glomerulonephritis	2	2	—
Subacute glomerulonephritis	5	5	1
Chronic glomerulonephritis	1	1	—
Malignant hypertension ..	2	2	—
Total	24	18	9

DISCUSSION

It is notable that the antisera obtained from all five rabbits injected with human gamma globulin cross-reacted with human albumin. This may have been due to electrophoretically undetectable amounts of albumin contaminating the gamma globulin preparation, or to the presence of common antigenic groupings by the two protein fractions. The fluorescent antibody stain was capable of reacting against these two and possibly more serum protein components, and did not stain specifically for human gamma globulin. Similar difficulty in the preparation of specific antisera to apparently homogeneous human serum protein fractions has been experienced by others (Goodman *et alii*, 1956).

The absence of fluorescence from the lnmen of vessels other than the glomerular capillaries, the capsular space, the tubular lumen (except for casts), and the tubular epithelium, which is known to reabsorb protein from the glomerular filtrate (Oliver, 1948; Spector, 1954), suggests that the majority of the unbound serum protein was washed out in the preparation of the sections. Thus, the presence of fluorescence indicated a firm attachment of serum protein to the glomerular capillary walls.

Proteinuria was present without glomerular fluorescence in ten cases, and glomerular fluorescence occurred without proteinuria in one case. In nine cases, glomerular damage of various kinds was unassociated with fluorescence. Thus, it appears that the binding of fluorescent antibody was not directly related to the presence of either proteinuria or glomerular damage *per se*. Active membranous glomerulonephritis

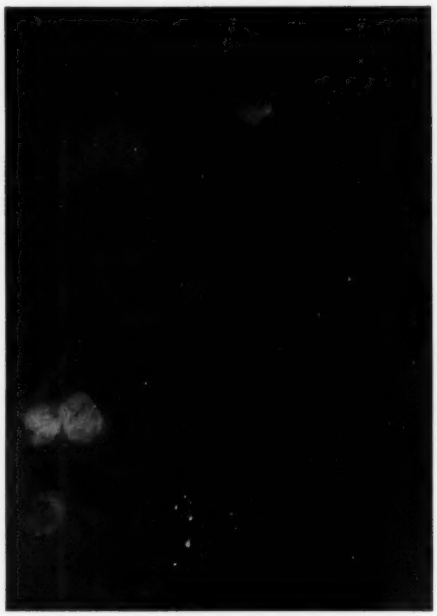

FIGURE XI

Kidney biopsy in Case No. 20 with chronic glomerulonephritis, stained with fluorescent antibody, showing fluorescence of casts in the tubules. A non-fluorescing scarred glomerulus is also faintly outlined at the top left-hand portion. ×200.

was observed in eight of the nine cases in which the glomeruli fluoresced. The nephritis was associated with D.L.E. in five cases, lupoid hepatitis in one case, arthritis (but no L.E. cells) in one case and *diabetes mellitus* in one case. Thus, in the present group of biopsies the localization of serum proteins in the glomeruli was most frequently associated with the membranous glomerulonephritis occurring in cases of D.L.E.

One fatal case (18) of glomerulonephritis showed fluorescence similar to that found by Mellors and Ortega (1956); however, glomeruli

from two patients (16, 17) with acute glomerulonephritis failed to fluoresce, possibly because the biopsies were taken in the recovery phase. No fluorescence was observed in the glomeruli of patients with chronic glomerulonephritis.

FIGURE XII

Glomerulus from kidney biopsy in Case No. 8, a female aged 29 years with nephrotic syndrome and positive L.E. cell findings. There is irregular basement membrane thickening, endothelial hyperplasia, capsular adhesion and periglomerular fibrosis. H. & E. ×400.

Mellors and Ortega (1956), using a specific fluorescein-coupled antihuman gamma globulin on post-mortem material, claimed that the localization of gamma globulin on the glomerular capillary walls in acute glomerulonephritis, lipid nephrosis and *periarteritis nodosa* indicated an antigen-antibody reaction occurring at this site. Joske and Stubbe (1957) suggested that the periodic acid-Schiff staining of the thickened basement membrane in the glomeruli of patients with lupus nephritis was due to the hexosamine content of gamma globulin specifically absorbed to this site.

Recently, evidence that immunological anomalies occur in cases of D.L.E. and lupoid hepatitis was presented by Gajdusek (1958) and Mackay and Gajdusek (1958), who found circulating complement-fixing antibodies to human tissue antigens in the sera of these patients. They considered that the presence of these antibodies might reflect a more fundamental anomaly of cell-bound antibody. Because of the striking evidence of the binding of serum protein to the glomerular capillary walls in this particular group of cases we believe that it may represent gamma globulin localization as a manifestation of an antigen-antibody reaction.

An alternative hypothesis is the secondary permeation of the damaged capillary wall of the glomeruli by plasma proteins. Thus Gitlin *et alii* (1957), utilizing fluorescent antibody prepared against human fibrinogen, located fibrin in the glomerular capillaries of post-mortem kidney sections from cases of D.L.E. The morphology and staining qualities of the thickened capillary wall may be due in part to the non-specific adsorption of plasma proteins.

FIGURE XIII

Glomerulus from kidney biopsy in Case No. 8 stained with fluorescent antibody, showing strong fluorescence of glomerular capillaries and capsule. Some casts are present in the tubules in the upper left-hand corner. A glomerulus with slight fluorescence is present at the top. ×350.

This could be the sole explanation of the weak positive fluorescence obtained in the glomeruli of the diabetic patient, where the lesion was probably metabolic in origin. Such a hypothesis does not invalidate the concept of a localized antigen-antibody reaction, but the specific localization of gamma globulin would be masked by the non-specific secondary adsorption of plasma proteins.

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CONGENITAL DEFICIENCY OF FACTOR VII[1]

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SUMMARY

Laboratory investigations are described in a female patient who suffers from a congenital hæmorrhagic disorder due to deficiency of Factor VII. The one-stage prothrombin time using brain extract was greatly prolonged, but using viper venom was normal. All tests of the intrinsic blood coagulation mechanism, including the thromboplastin generation test, were normal. The patient provides the eighth recorded case of Factor VII deficiency with this pattern of laboratory findings.

Evidence is presented which supports the concept that Factor VII consists of at least two components, deficiency of either producing a prolonged one-stage prothrombin time using brain extract. Cases reported in the literature of Factor VII deficiency associated with an abnormal thromboplastin generation test and prolonged plasma venom time probably represent deficiency of the second component. The Factor VII component deficient in this patient is reduced early in therapy with " Dindevan ".

The patient has three children, all with reduced concentrations of Factor VII. It is considered that the children represent the heterozygous state of Factor VII deficiency and that the defect is transmitted in a recessive manner.

OWEN AND BOLLMAN (1948) showed that the addition of serum would accelerate the formation of thrombin in the plasma of dogs treated with dicoumarin. A similar property of normal human serum was investigated by Koller *et alii* (1951), who demonstrated that serum acts as an accelerator for the conversion of prothrombin to thrombin in the presence of tissue extract and calcium. These authors considered that the accelerating property of serum was due to the presence of a specific substance which they named Factor VII. Factor VII was reduced in concentration in the serum of patients receiving dicoumarin, was stable on storage and was adsorbed by calcium phosphate, barium suphate and Seitz filtration. Since 1951, the prolonged one-stage prothrombin time observed, using tissue extracts as a source of thromboplastin, during therapy with anticoagulant drugs has been attributed largely to deficiency of Factor VII.

Factor VII has remained an ill-defined blood-clotting substance. Activity in plasma is much less than in serum, so that most experiments which claim to demonstrate the properties of this factor have been carried out using serum. Such experiments often present considerable difficulties in interpretation. Several clotting factors have now been described as occurring in normal serum, and as these have very similar physico-chemical properties, their isolation has proved impractical. An illustration of the difficulty is the confusion that exists over the role of Factor VII in intrinsic blood coagulation mechanisms. Biggs and Douglas (1953), using the newly developed thromboplastin generation test, found defective thromboplastin generation in systems containing sera from patients receiving anticoagulant drugs. It was thought then that prothrombin and Factor VII were the only substances reduced in concentration in the blood of these patients, and the authors concluded that Factor VII was essential for normal blood thromboplastin generation. Subsequent work involving observations on the reactions of mixtures of pathological sera in the thromboplastin generation test indicated that blood samples from patients receiving dicoumarin, " Tromexan " or " Dindevan " were frequently deficient in Christmas factor (Sise *et alii*, 1955 ; Douglas, 1955), and Factor X (Duckert *et alii*, 1955). These latter substances are involved in blood thromboplastin production, Christmas factor affecting the yield (Biggs *et alii*, 1952) and Factor X, the rate of production. The reactions ascribed to Factor VII deficiency could have resulted from deficiency of these other factors. Blood samples from some patients on " Dindevan " therapy associated with prolonged one-stage prothrombin times may react normally in the thromboplastin generation test (Hicks and Pitney, 1957).

It was hoped that observations on blood samples from patients suffering from congenital deficiency of Factor VII would assist in defining

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the function of this factor in normal blood coagulation. Since 1950 several case reports have been published of patients suffering from a congenital hæmorrhagic disorder manifested by a prolonged one-stage prothrombin time which was corrected by the addition of normal serum to the patient's plasma. However, these cases have not all presented uniform findings. Seven of the patients (Koch *et alii*, 1955, (Case 1); Hicks, 1955; Owren *et alii*, 1955; Jürgens, 1956; Ackroyd, 1956; Alexander, 1956; Dische, 1957) have had greatly prolonged one-stage prothrombin times, yet have had no detectable abnormality in the intrinsic blood coagulation mechanism; in particular, the thromboplastin generation test has given normal results. Alexander's patient had been previously described by Alexander *et alii* (1951) as an example of SPCA deficiency, Ackroyd's patient by Jenkins (1954) as Factor VII deficiency, and Owren, Newcomb and Hjort's patient by Owren (1953) as hypoproconvertinæmia. The clotting defect was reevaluated by the later authors using the thromboplastin generation test. Other tests of clotting function have also been normal. In these patients blood clots normally under the influence of its own coagulation mechanism; the only detectable abnormality is that prothrombin is converted slowly to thrombin in the presence of tissue extracts and calcium. It is difficult to understand why these patients suffer from a hæmorrhagic tendency, as tissue extracts are not normally thought to play any part in in-vivo hæmostasis.

A much larger group of patients have been considered to be suffering from congenital Factor VII deficiency in whom there has been evidence of a serious derangement of the coagulation mechanism. Like the previous group, these patients have had a prolonged one-stage prothrombin time corrected by the addition of normal serum to the plasma; in addition they have had other abnormalities, such as prolonged whole-blood coagulation times, diminished consumption of prothrombin during coagulation and abnormal thromboplastin generation tests.

The attempted correlation of the findings in these two groups of patients has brought further confusion to the concept of Factor VII as a separate clotting factor. Ackroyd (1956) pointed out that it was unlikely that the differences in laboratory findings could be explained on degrees of deficiency of Factor VII. The patients with normal thromboplastin generation tests have often had considerably longer prothrombin times than those with other coagulation defects. Several of the patients with severe abnormalities in the thromboplastin generation test have had only moderately prolonged prothrombin times. Some of these patients may suffer from multiple deficiencies of clotting factors, and Biggs (1956) has provided suggestive evidence of this on the results of mixing experiments in the thromboplastin generation test. The patients of Telfer *et alii* (1956) and Hougie *et alii* (1957) are unusual in that the prolonged one-stage prothrombin time was corrected by normal serum and also by serum samples from some patients on "Dindevan" therapy. Factor VII is considered to be deficient in the blood of patients receiving "Dindevan". The patients' sera gave an abnormal result in the thromboplastin generation test, presumably due to deficiency of a single clotting substance. Plasma samples from Alexander's patient and the patient of Hougie and co-workers were mutually corrective in the prothrombin time test.

The only reasonable conclusion seems to be that there are at least two substances present in normal blood and demonstrable in serum, deficiency of either resulting in a prolonged one-stage prothrombin time when tissue extract is used in the test. One of these substances is necessary for normal serum activity in the thromboplastin generation test; the other substance plays no part in thromboplastin generation. Both give to serum its "Factor VII" activity. As neither substance has been clearly identified, it seems premature at this stage to confuse the coagulation literature with additional terms such as Prower factor (Telfer *et alii*, 1956), Stuart factor (Hougie *et alii*, 1957) and Factor D (Biggs, 1956). In this paper it is proposed to retain the generic term "Factor VII deficiency" to indicate a prolonged one-stage prothrombin time, when the test is carried out with tissue extract, which is corrected by the addition of normal serum to the test plasma. When several case reports of congenital Factor VII deficiency present a uniform pattern of laboratory findings, the term will be modfied by a descriptive label relating to a key case. Thus of the seven patients described in whom there is no evidence of a defect in the intrinsic blood coagulation mechanism, that of Ackroyd (1956) has been investigated most clearly, and these patients will be referred to as suffering from Factor VII deficiency (Ackroyd type). A further example of this condition is described below. The patient has three children, and a family study provides evidence of the mode of inheritance of this deficiency.

CASE REPORT

The patient is a Dutch migrant, aged 32 years, who was first examined in the laboratory in May, 1956, eight weeks after her arrival in Australia. She was admitted to hospital because of a large hæmatoma of the thigh which had developed after a fall at her home on the previous day.

She has suffered from a bleeding tendency all her life, and has been transfused an estimated total of 50 pints of blood. She bled for two to three weeks after each dental extraction, and transfusions were usually necessary. She has had three live children only from eight deliveries at term, being told that the five stillbirths were due to Rhesus incompatibility. Postpartum hæmorrhage was usually severe enough to require transfusion.

She bruises easily following slight injury, but has never suffered from hæmarthroses or gastro-intestinal hæmorrhage. She leads a fairly normal active life.

Her three living children are all girls, aged eight, six and two years. The girl aged six suffered from hæmolytic disease of the newborn and was transfused at birth; the parents feel that she is a little backward at school, but has no other sequelæ of kernicterus. None of the children shows an unusual tendency to bleed. The patient's parents and siblings live in Holland. She has brothers and sisters with no bleeding tendency. There is no history of abnormal bleeding in either of her parents or their families. Her mother and father are not blood relations.

Physical examination disclosed a healthy obese woman with a large hæmatoma of the thigh, but no other abnormal findings except for several carious teeth. She was discharged from hospital after two days, and the hæmatoma subsided uneventfully. During an 18-month period of observation she has kept well. She was recently readmitted to hospital for the extraction of four carious teeth.

500 millilitres of fresh plasma were infused intravenously during one hour prior to extraction. The four teeth were extracted under local anæsthesia, gauze soaked in topical thrombin solution (Parke-Davis) was laid over the sockets, and a dental splint was fitted. A further 1000 millilitres of fresh plasma were infused during the three hours subsequent to the extractions and a further 500 millilitres over the next 12 hours. Two litres of plasma were therefore given over a 16-hour period. The patient received no further therapy. There was no excessive bleeding from the tooth sockets, the dental splint was removed on the third day, and the patient was discharged from hospital on the sixth day.

Family Blood Group Serology

The patient is blood group A Rhesus-negative, and her serum contains incomplete anti-D antibodies to a titre of 1 : 32 estimated by the indirect Coombs reaction. The husband is Rhesus-positive. The children of eight and six years are both Rhesus-positive. The child of two years is Rhesus-negative.

LABORATORY INVESTIGATION OF THE PATIENT'S HÆMOSTATIC MECHANISM

Unless otherwise stated, all tests of clotting function were carried out as described by Biggs and Macfarlane (1957). Prothrombin time estimations were performed on plasma obtained by centrifuging a mixture of nine volumes of venous blood and one volume of 3·8% sodium citrate solution. Tissue extract for one-stage prothrombin time estimations was prepared from acetone-dried human brain. Prothrombin times were also carried out using Russell viper venom ("Stypven"). The venom solution was used at a dilution of 1 : 10,000. To a mixture of 0·1 millilitre of plasma and 0·1 millilitre of venom solution warmed to 37° C. was added 0·1 millilitre of previously warmed M/40 calcium chloride. The time taken for the mixture to clot was recorded as the venom time. Venom-lipoid times were estimated by adding 0·1 millilitre of a 1% solution of crude cephalin prepared from human brain to the mixture of plasma and venom before recalcification.

It was necessary to modify the described techniques for estimating prothrombin consumption, because of the greatly prolonged prothrombin time of the patient's plasma using brain extract. The prothrombin activity of plasma and serum tested one hour after coagulation were compared in the following manner: to 0·1 millilitre of patient's plasma (or serum) was added 0·1 millilitre of normal serum, 0·1 millilitre of brain extract and 0·1 millilitre of M/40 calcium chloride, all reagents being at 37° C. Exactly 60 seconds after adding the calcium chloride, 0·2 millilitre of warmed human fibrinogen solution (200 milligrammes per 100 millilitres) was added to the mixture and the speed of clotting of the fibrinogen determined. When plasma was tested, it was necessary to remove the initial clot which formed in about 12 seconds with an applicator stick before adding the fibrinogen solution. The normal serum used in this test was allowed to remain in contact with the clot at 37° C. for four hours before use.

The thrombin generation test was performed on platelet-rich plasma as described by Pitney and Dacie (1953).

Thromboplastin generation in the patient's blood was tested by the method of Biggs and Douglas (1953), except that a lipoid material prepared from human brain (Bell and Alton, 1954) was used as a substitute for platelet suspensions. Thromboplastin generation was also tested by the method of Hicks and Pitney (1957).

The patient's plasma anti-hæmophilic globulin (AHG) concentration was estimated by the method of Pitney (1956).

The concentrations of serum factors necessary for normal thromboplastin generation were estimated by observing the ability of dilutions

of the patient's serum to correct the abnormal thromboplastin generation shown by a serum sample from a patient receiving "Dindevan" therapy. The degree of correction was compared to that shown by normal serum and serum from a patient with Christmas disease. These tests were modelled on similar procedures described by Biggs (1956).

Concentrations of Factor VII in plasma samples from the patient's husband and three children were estimated in the following manner: dilutions of normal plasma in the patient's plasma from 2% to 50% were made, and the one-stage prothrombin time using brain extract was recorded for each mixture. A series of four dilutions of each plasma under test was also made in the patient's plasma, and the one-stage prothrombin times were recorded. These were compared with the times given by the normal plasma dilutions and the average concentration calculated for each plasma.

Results

The patient has been investigated in the laboratory several times over an 18-month period, and the following results are representative of several observations. The results of the more usual coagulation tests are shown in Table I. The result of the tourniquet test, the bleeding time, the coagulation time, clot retraction and the platelet count were all normal. The one-stage prothrombin time using brain extract was greatly prolonged.

TABLE I

Results of Preliminary Tests of the Hæmostatic Mechanism

Test	Result in Patient	Normal Range
Tourniquet test	Negative	—
Whole-blood coagulation time (Lee-White)	8½ minutes	5–11 minutes
Clot retraction	Good	—
Bleeding time (Duke)	5 minutes	0–7 minutes
Platelet count	250,000 per cubic millimetre	150,000–300,000 per cubic millimetre
One-stage prothrombin time	77 seconds	12–14 seconds

Thrombin Generation Test.—The thrombin generated in the patient's platelet-rich plasma after recalcification was studied by subsampling from the mixture at one-minute intervals into a solution of human fibrinogen. The pattern of thrombin production has been normal repeatedly. Some typical data are shown in Table II.

Prothrombin Consumption Test.—When brain extract and calcium chloride were added to a mixture of equal volumes of the patient's plasma and normal serum, the mixture contained sufficient thrombin after 60 seconds' incubation to clot the added fibrogen in six seconds. When the patient's serum tested one hour after clotting was substituted for the patient's plasma, the fibrinogen clotting time was 92 seconds. The prothrombin consumption index (plasma fibrinogen clotting time as a percentage of serum fibrinogen clotting time) was 7%. Normal plasma and serum tested in an identical fashion gave a plasma fibrinogen clotting time of eight seconds and a serum fibrinogen clotting time of 37 seconds (index 21%). It was concluded that prothrombin in the patient's blood was consumed normally during in-vitro coagulation.

TABLE II

Thrombin Generation Test[1]

Plasma	Time in Minutes after Recalcification						
	½	1½	2½	3½	4½	5½	6½
Normal	—	—	25	14	17	26	51
Patient's	—	240	12	11	17	42	—

[1] 0·05-millilitre volumes of recalcified platelet-rich plasma were added to 0·2-millilitre volumes of fibrinogen solution at one-minute intervals. The figures refer to the clotting times of the fibrinogen in seconds.

TABLE III

Thromboplastin Generation Test[1]

$Al(OH)_3$-Treated Plasma Diluted 1:5	Serum Diluted 1:10	Clotting Time (in Seconds) of Substrate Plasma after Incubation for Periods Stated (Minutes)					
		1	2	3	4	5	6
Normal	Normal	31	13	10	10½	10½	10½
Patient	Patient	31	11	8½	9	9	—

[1] The incubation mixture consisted of equal volumes of diluted $Al(OH)_3$-treated plasma, diluted serum, lipoid platelet substitute and M/40 calcium chloride.

Thromboplastin Generation Test.—Thromboplastin generation in the patient's blood has been normal repeatedly when tested by either the classical method (Biggs and Douglas, 1953) or the screening method (Hicks and Pitney, 1957). Representative results of the thromboplastin generation test are shown in Table III. Plasma thromboplastin generated by a mixture of the patient's $Al(OH)_3$-treated plasma, patient's serum and lipoid platelet substitute

TABLE IV

The Effect of Various Reagents on the Patient's Prolonged One-Stage Prothrombin Time

Patient's Plasma (ml.)	Saline (ml.)	Normal Serum (1 : 10) (ml.)	Patient's Serum (1 : 10) (ml.)	Normal Plasma (1 : 10) (ml.)	$Al(OH)_3$ Plasma (1 : 5) (ml.)	$Al(OH)_3$ Plasma (ml.)	Prothrombin Time (secs.)
0·1	—	—	—	—	—	—	41
0·05	0·05	—	—	—	—	—	55
0·05	—	0·05	—	—	—	—	12
0·05	—	—	0·05	—	—	—	39
0·05	—	—	—	0·05	—	—	17
0·05	—	—	—	—	0·05	—	40
—	—	—	—	—	—	0·1	78

was able to clot the patient's whole plasma in the normal time of seven seconds.

Investigation of the Prolonged One-stage Prothrombin Time.—The one-stage prothrombin time of the patient's plasma using brain extract has varied from 41 to 89 seconds over the period of observation, readings on most occasions being greater than 70 seconds. Over the same period, the prothrombin times of normal control plasma samples have been between 12 and 14 seconds. In Table IV are shown the prothrombin times of mixtures of the patient's plasma with various reagents, equal volumes of the plasma and reagent being used. The addition of an equal volume of a 1 : 10 dilution of normal serum to the patient's plasma resulted in a mixture with a prothrombin time of 12 seconds. Normal serum is deficient in both prothrombin and Factor V, and the normal prothrombin time of the mixture suggests that the patient's plasma contained adequate amounts of these substances. $Al(OH)_3$-treated normal plasma contains Factor V, but the addition of this reagent to the patient's plasma did not appreciably shorten the prothrombin time. This also suggests that the patient's plasma was not deficient in Factor V.

The experiments so far reported confirm that the patient suffers from a deficiency of a serum clotting factor necessary for the conversion of prothrombin to thrombin in the presence of tissue extract and calcium. The intrinsic blood coagulation mechanism as evidenced by bleeding and clotting times, prothrombin consumption, thrombin and thromboplastin generation tests is normal.

Plasma samples were obtained from five patients receiving " Dindevan " therapy. Three patients had been receiving the drug for three days only ; the other two had had therapy for three and four weeks. The one-stage prothrombin times using brain extract ranged from 26 to 55 seconds. Prothrombin times were performed on mixtures of equal volumes of the patient's plasma and each " Dindevan " plasma. Four mixtures (including plasma samples from the three patients who had recently commenced " Dindevan " therapy) showed no significant shortening of the prothrombin time. A mixture of one " Dindevan " plasma, obtained after four weeks' drug therapy, and the patient's plasma showed considerable mutual correction. Mixing experiments involving plasma and serum samples from this patient (P), the patient with congenital Factor VII deficiency, and from a patient (S) on " Dindevan " for three days are shown in Table V. The prothrombin time of plasma P was 26 seconds and that of plasma S 35 seconds, while a mixture of equal volumes of these plasmas showed a prothrombin time of 18 seconds. A mixture of equal volumes of plasma P and plasma from the patient with Factor VII deficiency had a prothrombin time of 16 seconds. Plasma S and the Factor VII-deficient plasma were not mutually corrective.

Serum P almost completely corrected the prothrombin time of the Factor VII-deficient patient, while serum S was much less effective. Normal serum was effective in correcting the prothrombin time of both plasma S and the Factor VII-deficient plasma. These experiments suggest that plasma S and the Factor VII-deficient plasma had the same defect. Plasma P contained an appreciable quantity of Factor VII. The prolonged prothrombin time shown by this plasma could have been due mostly to prothrombin deficiency.

The one-stage prothrombin time of the patient's plasma using brain extract has shown a consistent tendency to become shorter on storage in contact with glass. In one such experiment the patient's plasma, which had an *i*nitial prothrombin time of 77 seconds, was

TABLE V

One-Stage Prothrombin Times of Mixtures of Patient's Plasma and Plasma and Serum Samples from Two Patients on "Dindevan" Therapy[1]

Patient's Plasma (ml.)	Plasma S (ml.)	Plasma P (ml.)	Serum S (ml.)	Serum P (ml.)	Normal Serum (ml.)	Prothrombin Time (secs.)
0·1	—	—	—	—	—	89
—	0·1	—	—	—	—	35
—	—	0·1	—	—	—	26
—	0·05	0·05	—	—	—	18
0·05	0·05	—	—	—	—	38
0·05	—	0·05	—	—	—	16
0·08	—	—	0·02	—	—	30
0·08	—	—	—	0·02	—	13
0·08	—	—	—	—	0·02	12
—	0·08	—	—	—	0·02	13

[1] Samples S were obtained from a patient on "Dindevan" therapy for three days; samples P were from a patient receiving "Dindevan" for four weeks.

left in an unsiliconized glass tube at 4° C. for six hours. The prothrombin time was reduced to 41 seconds. After two days' storage at 4° C. it was 34 seconds. The prothrombin time of the control plasma remained at 12 seconds throughout.

Prothrombin Time Using Viper Venom.—The venom time of the patient's plasma was normal. A mixture of plasma, venom solution and calcium chloride clotted in 12·5 seconds (normal control plasma 13·5 seconds). When cephalin was added to the plasma-venom mixture before recalcification, the clotting time was shortened to five seconds (normal control plasma six seconds).

Concentration of Thromboplastin-Precursor Substances.—The AHG concentration of the patient's plasma was estimated to be 165% of the standard normal plasma (normal range 50% to 220%).

The ability of the patient's serum to correct the abnormal thromboplastin generation of serum from a patient on "Dindevan" therapy is shown in Table VI. The patient receiving "Dindevan" had a plasma prothrombin time using brain extract of 105 seconds. His serum when tested with normal $Al(OH)_3$-treated plasma and lipoid platelet substitute in the thromboplastin generation test produced an abnormal result. A mixture of nine parts of "Dindevan" serum and one part of normal serum produced nearly normal thromboplastin generation, as also did a mixture of nine parts of "Dindevan" serum and one part of patient's serum. Serum from a patient with Christmas disease was not as effective as normal serum in correcting the abnormal thromboplastin generation of "Dindevan" serum. This indicates that the "Dindevan" serum was deficient in Christmas factor. The partial correction by Christmas disease serum suggests that the "Dindevan" serum was also deficient in other factor(s) which were supplied by the Christmas disease serum.

TABLE VI

Thromboplastin Generation Tests using Various Sera and Serum Mixtures at a Dilution of 1 : 10[1]

Type of Serum or Serum Mixture	Clotting Time (in Seconds) of Substrate Plasma after Incubation for Periods Stated (Minutes)					
	1	2	3	4	5	6
Normal	—	—	18	12	10	10
"Dindevan" ..	—	—	42	39	31	30
"Dindevan", 9 parts; normal, 1 part ..	—	—	16	12	11	11
"Dindevan", 9 parts; patient, 1 part ..	—	—	20	14	12	12
"Dindevan", 9 parts; Christmas disease, 1 part	—	—	18	16	16	16

[1] The incubation mixtures consisted of equal volumes of diluted normal $Al(OH)_3$-treated plasma, the diluted serum or serum mixture being tested, lipoid platelet substitute and M/40 calcium chloride.

The normal behaviour of serum from the patient with Factor VII deficiency indicates a normal concentration of serum factors necessary for thromboplastin production.

Family Study

The one-stage prothrombin time of plasma from the patient's husband was 14 seconds, the same as that of a normal control tested concurrently. The prothrombin times of plasma

samples from the three children were all 15·5 to 16 seconds. Quantitative Factor VII assay using the patient's plasma as diluent showed the concentration in the husband's plasma to be 75% to 100% of the normal control and the three children all 30% to 40% of normal.

DISCUSSION

The laboratory findings of the patient reported in this paper appear to be identical with those of the patients of Koch *et alii* (1955), Hicks (1955), Owren *et alii* (1955), Jürgens (1956), Ackroyd (1956), Alexander (1956) and Dische (1957). In all, the prolonged one-stage prothrombin time of plasma was corrected by the addition of normal serum. In all, the thromboplastin generation test has given normal results, and other tests of clotting function have been normal also. These eight patients appear to be the only certain examples of Factor VII deficiency (Ackroyd type) reported so far. In five patients the plasma venom time was carried out and was found to be normal.

The paucity of well authenticated cases is due partly to the fact that the concept of Factor VII has only been firmly established since the publication of Koller *et alii* (1951). The thromboplastin generation test was not described until 1953. This is the most sensitive test available for the study of the intrinsic blood coagulation mechanism, and it is difficult to classify case reports of Factor VII deficiency which do not include the result of this test. The justification for separation of the Ackroyd type of Factor VII deficiency from the larger group of patients with prolonged prothrombin times corrected by serum is that there is no demonstrable defect in the intrinsic blood coagulation mechanism in the Ackroyd type. Other tests of clotting function, such as whole-blood coagulation time and prothrombin consumption, are so insensitive that they may give normal results in the presence of serious derangement of the hæmostatic mechanism. Without information concerning the results of the thromboplastin generation test, any attempt at classification of published cases must be on the basis of probability only.

Giordano (1943) described a case of idiopathic hypoprothrombinæmia in which the prolonged plasma prothrombin time was corrected by the addition of normal serum to the patient's plasma. Bleeding and coagulation times were normal, but no further relevant information is available. Crockett *et alii* (1949) also reported on a patient with hypoprothrombinæmia. The prolonged prothrombin time was corrected by normal serum. This case has been reinvestigated by Hougie *et alii* (1957), who found that the thromboplastin generation test and plasma venom time were both abnormal The patient of Landwehr *et alii* (1950) probably suffered from Factor VII deficiency, but laboratory evidence is insufficient.

In publications since 1950 the hæmostatic mechanism has been investigated more fully, and different patterns of laboratory findings become apparent. Frick and Hagen (1953) described a patient with deficiency of the stable prothrombin conversion factor. The plasma prothrombin time was prolonged, but was completely corrected by the addition of an equal volume of normal serum. Both the bleeding and coagulation times were clearly prolonged. Lewis *et alii* (1953) described two cases of congenital hypoproconvertinæmia associated with prolonged coagulation times and abnormal prothrombin consumption. One of these patients (R.S.) has been restudied by Hougie and co-workers (1957), who found an abnormal response to the thromboplastin generation test and a prolonged plasma venom time. The patient of Beaumont and Bernard (1953) showed prolonged bleeding and coagulation times and abnormal prothrombin consumption. Hule *et alii* (1955) described a newborn infant with prolonged bleeding and coagulation times but with normal prothrombin consumption. In all these patients, the prothrombin time was prolonged, and correction was effected by the addition of normal serum; the presence of other abnormal coagulation findings makes it unlikely that any suffered from the Ackroyd type of deficiency.

On the other hand the two patients of Long *et alii* (1955), the two patients of Quick *et alii* (1955) and the patient of Choremis *et alii* (1956) may have suffered from the type of Factor VII deficiency exemplified by the patient reported here. In all a detailed coagulation study did not reveal any defect apart from a prolonged prothrombin time. However, the thromboplastin generation test was not employed.

The three patients of Bell and Alton (1955), the three of de Vries *et alii* (1955), the two of Chevallier *et alii* (1955), the two of Stefanovic *et alii* (1955), the twins of Biggs (1956) and the patients of Soulier *et alii* (1955), Greig and Tattersall (1956, Case 5), Greig *et alii* (1956), Newcomb *et alii* (1956) and Telfer *et alii* (1956) all showed abnormal serum reactions in the thromboplastin generation test and do not correspond to the Ackroyd type of Factor VII deficiency. It is probable that some of these

patients suffered from multiple deficiencies of clotting factors. Biggs (1956) reexamined the case of Newcomb and co-workers and reported deficiencies of Factor X and Christmas factor as well as Factor VII. She also examined blood samples from Jürgens's and Telfer's patients and found them to have different defects.

Koller investigated blood samples from a patient of Chevallier and co-workers and found deficiency of Factor X as well as Factor VII, but the concentration of Christmas factor was normal.

The reactions given by serum samples in the thromboplastin generation test from patients with Factor VII deficiency appear to be the most reliable means of separating the reported cases into groups. A review of the literature with inclusion of the present case shows that the thromboplastin generation test has been carried out on 27 patients. These include one pair of identical twins (Biggs, 1956), two cousins (Chevallier *et alii*, 1955) and three members of one family (Bell and Alton, 1955), so that 23 families are represented. Assuming that the type of deficiency was similar in the one family, a representative case only from these three publications has been included in the data presented in Table VII. In this table the relevant features of the laboratory findings are compared.

It is difficult to compare many of the laboratory findings because of differences in laboratory technique and variations in the results given for normal controls. In some publications, the results of some tests are simply indicated as being normal without actual data

TABLE VII

Findings in 23 Patients with Factor VII Deficiency in Whom the Thromboplastin Generation Test was Performed

Authors	Sex	Blood Coagulation Time	Prothrombin Time (secs.). Normal control in brackets	Plasma Venom Time	Thromboplastin Generation Test	Concentration Christmas Factor	Concentration Factor X
Ackroyd	M	Normal	42–70 (12–16)	Normal	Normal	Probably Normal	—
Alexander	F	Normal	72 (17)	—	Normal	—	—
Bell and Alton ..	M	Prolonged	22	—	Abnormal	—	—
Biggs	F	Normal	29–42 (17)	Abnormal	Abnormal	50%	0%
Chevalier *et alii* ..	M	Prolonged	40–50 (12)	—	Abnormal	Normal	Deficient (Koller)
Crockett *et alii* ..	F	Prolonged	55 (14)	Abnormal (Hougie)	Abnormal (Hougie)	—	—
Dische	F	Normal	55–111 (11½–15)	Normal	Normal	—	—
Greig and Tattersall ..	—	Prolonged	67 (12)	—	Abnormal	Deficient	Deficient
Greig *et alii*	M	Normal	65–235 (12)	—	Abnormal	—	—
Hicks	F	Normal	45 (13)	Normal	Normal	—	—
Hougie *et alii* ..	M	Prolonged	60 (12)	Abnormal	Abnormal	Normal	Normal
Jürgens	M	Normal	less than 10%	—	Normal	100+% (Biggs)	40% (Biggs)
Koch *et alii*	F	Normal	50 (21)	—	Normal	—	—
Newcomb *et alii* ..	F	Prolonged	72 (15)	Abnormal	Abnormal	Trace only (Biggs)	0% (Biggs)
Owren *et alii* ..	F	Normal	55–60 (14)	Normal	Normal	—	—
Soulier *et alii*	F	Normal	29–85 (11)	—	Abnormal	Normal	—
Stefanovic *et alii* ..	M	Normal	26 (10)	—	Abnormal	—	—
	M	Prolonged	27 (13)	—	Abnormal	—	—
Telfer *et alii*	F	Normal	24–30 (9–12)	Normal once. Abnormal once (Biggs)	Abnormal	75% (Biggs)	100% (Biggs)
de Vries *et alii* ..	M	Prolonged	20 (15)	—	Abnormal	Probably normal	—
	M	Normal	17 (13)	—	Abnormal	Probably normal	—
	M	Prolonged	20 (15)	—	Abnormal	Deficient	—
Present case	F	Normal	41–85 (12–14)	Normal	Normal	Normal	Normal

Where a worker has restudied another author's patient and made an additional observation, the name appears in brackets with the observation

being presented. Of the 15 patients listed as having abnormal serum reactions in the thromboplastin generation test, nine were males and five females, and in one the sex was not recorded. The whole-blood coagulation time was reported as normal in six of the 15 patients. This emphasizes the fact that patients may have a disturbance of intrinsic thromboplastin production and still show a normal blood coagulation time. For this reason, no classification of types of Factor VII deficiency is possible on the results of coagulation times. The cases included in Table VII have been selected because all have been investigated with the thromboplastin generation test. Prothrombin times using brain extract in this group varied from 17 to 235 seconds. It is especially difficult to compare the results of prothrombin times, as the figures given for the normal controls varied from 9 to 17 seconds. The prothrombin time was less than 30 seconds in at least one test in nine of the 15 patients. The plasma venom time is reported for five patients only. In all five an abnormal result was obtained at least once; in the patient of Telfer and co-workers, the venom time was also reported as being normal once. The concentration of thromboplastin precursor substances was studied to some degree in 10 patients. In six a deficiency of either Christmas factor or Factor X or both was found.

Of the eight patients described with normal thromboplastin generation tests, six were females and two males. No patient had a prothrombin time less than 30 seconds. The viper venom time is recorded in five patients and was normal in all. Concentration of Christmas factor was found to be normal in the three patients in which this was investigated. Biggs reported a Factor X concentration of 40% in the serum of Jürgens's patient. This evidence of partial deficiency is questionable, as the tests were carried out on a stored serum sample, and Koller (1955) has shown that Factor X is labile on storage.

From the available evidence it appears that patients with Factor VII deficiency of the Ackroyd type have generally longer prothrombin times than the group with abnormal thromboplastin generation tests. The reaction of plasma to viper venom may also be important in separating the two groups. In this connexion, it is very important to have verification as to whether the plasma venom time was abnormal in the patient of Telfer *et alii* (1956). This patient had an abnormal thromboplastin generation test. In the original publication, the plasma venom time was reported as normal. Biggs (1956) restudied this patient and found an abnormal venom time.

The conclusions of Rapaport *et alii* (1954), that proconvertin deficiency can be diagnosed on the finding of a prolonged prothrombin time using brain extract but a normal venom time, should be modified. The plasma of patients with Factor VII deficiency, such as the one described in this paper, shows an abnormal prothrombin time with brain extract, but a normal time with Russell viper venom. Rapaport and co-workers were of the opinion that a prolonged prothrombin time with both brain and venom indicated either prothrombin or Factor V deficiency. However, Factor VII deficiency of the type described by Hougie *et alii* (1957) also gives abnormal results with both brain extract and viper venom.

Inferences drawn from the prothrombin times of mixtures of plasma or serum samples from patients with Factor VII deficiency and patients receiving anticoagulant drugs must be accepted with caution. The coagulation defect in the blood of patients receiving these drugs is complex. As well as prothrombin and Factor VII deficiency (of more than one type), there is a variable deficiency of both Christmas factor and Factor X. The degree of Factor VII deficiency has been based on the corrective effect of normal serum. Most of the case reports have shown no mutual correction in mixtures of plasma from patients with Factor VII deficiency and patients receiving anticoagulant drugs. However, both Telfer *et alii* (1956) and Hougie *et alii* (1957) found that samples from patients recently started on "Dindevan" therapy would correct the deficiency in their patients. The corrective effect diminished after prolonged "Dindevan" therapy. In the case reported here, four plasma samples from patients on "Dindevan" therapy for only three days did not correct the plasma defect, whereas one of two samples collected after prolonged "Dindevan" therapy was able to achieve considerable correction. If it is accepted that there are two components to what is generally considered to represent Factor VII, then the Ackroyd type of deficiency would appear to occur early in "Dindevan" treatment and the other type at a later stage. This agrees with the finding that the thromboplastin generation test is frequently normal when the blood of patients recently started on "Dindevan" is tested, even though the prothrombin time may be greatly prolonged.

Inheritance of Factor VII Deficiency

Several authors have reported family studies in their case reports of Factor VII deficiency and have drawn conclusions concerning the genetic inheritance of this defect. In many, the family studies have been very incomplete, and the evidence for defects in other members of the family has been based on slight prolongations of the prothrombin times in children, parents or siblings.. A normal prothrombin time has been taken to indicate a normal concentration of Factor VII. The picture is further confused because, as indicated in this paper, the coagulation defects have not been the same in all the reported cases. The question of inheritance can best be dealt with by considering first the eight case reports of Factor VII deficiency known to be of the Ackroyd type.

In Ackroyd's patient, no laboratory examination of relatives was possible. The patient was born of a consanguineous marriage, the patient's father and maternal grandmother being first cousins. There was a history of epistaxis amongst relatives on both the mother's and father's side of the family. Jürgens's and Hicks's patients had no clinically affected relatives, and laboratory studies were not carried out.

In the case reported here, the patient's husband had a normal Factor VII concentration and all three children had reduced concentrations of the order of 30% to 40% of normal. The patient's parents live in Holland and have not been examined. This evidence suggests that the deficiency is transmitted in a recessive manner, the patient being a homozygote deficient for Factor VII and all her children being heterozygotes, having obtained one normal gene for Factor VII from the father.

Laboratory studies on relatives of Owren's patient also suggest inheritance of a recessive characteristic. The patient's parents were not studied, but the father's brother had a proconvertin concentration of 27%. Proconvertin values in the patient and a clinically affected sister were from 1% to 3% of normal, whereas two brothers had values of 80% to 90% of normal. The patient's three children had proconvertin values between 14% and 27%. Proof of heterozygosity in both mother and father would be necessary to be certain that the defect was transmitted as a recessive characteristic. The mother of the patient of Koch and co-workers suffered from menorrhagia, and her blood showed a Factor VII concentration of 60%. No laboratory studies were carried out on the relatives of Alexander's patient. The mother and mother's brother bruised easily. The mother's father died of intestinal hæmorrhage after trauma.

The mother and father of Dische's patient were first cousins. There is clear evidence of abnormality in the patient's father, the plasma concentration of Factor VII being 24% to 35%. However, no abnormality could be detected in the mother. The patient's brother is a severely affected bleeder with evidence of Factor VII deficiency, and the patient's two children both have Factor VII concentrations between 24% and 35%. The only evidence against transmission of a recessive defect in this family is the normal Factor VII concentration in the patient's mother. However, it is possible that all heterozygotes would not have clearly abnormal laboratory findings. The degree of expression in laboratory tests of the abnormal gene in a heterozygote is probably related to the concentration characterizing the paired normal gene, and no information is available concerning normal variations in Factor VII concentration in the population. The other evidence in the family of Dische's patient is strongly in favour of transmission of a defective recessive gene. The only other possibility is that Factor VII deficiency is transmitted in autosomal dominant fashion, a patient requiring only one abnormal gene to manifest the disease. This seems most unlikely as it would be necessary to assume that relatives with no symptoms but slightly prolonged prothrombin times showed incomplete penetrance of the defective gene. There has not been a reported instance of a severely affected parent giving birth to a severely affected child, and this is the usual pattern in diseases transmitted by a single dominant gene.

In two of the eight patients reported as suffering from Factor VII deficiency of the Ackroyd type, the parents have married their cousins. Consanguinity makes it possible for both parents to have the same recessive gene.

Family studies in other probable cases of Factor VII deficiency of the Ackroyd type also indicate transmission of the defect as a recessive characteristic. The two patients of Long and co-workers were sisters. They were members of a large family, and had no other clinically affected relatives. The prothrombin time was prolonged in the patients' mother and maternal aunt and in four brothers and four sisters. A normal value was obtained in one brother. It is not clear from the authors' paper if the father was examined. Thus of 11 children, two could be considered homozygotes for Factor VII deficiency, eight were heterozygotes

with no clinical evidence of bleeding, and one was normal. The mother was a heterozygote. This distribution would fit the transmission pattern of a recessive gene if it is assumed that the father was a heterozygote.

The father of the patient described by Choremis *et alii* was the only other member of the family studied. He had no clinical symptoms, but a Factor VII concentration on assay of 50%. The two patients of Quick and co-workers both provide evidence for recessive transmission of the defect. Quick states that the prothrombin time of normal plasma in his laboratory is always 12 seconds, and he attaches considerable importance to very small deviations from this figure. The maternal grandfather of the patient in his first case had a prothrombin time of 14 seconds, suggesting heterozygosity. The maternal grandmother had a normal prothrombin time of 12 seconds. The patient's mother was a heterozygote with a prothrombin time of 14 seconds, and her husband, who was not a blood relation, also had a prothrombin time of 14 seconds. The patient's prothrombin time was 70 seconds, and her sister (a heterozygote) had a prothrombin time of 14 seconds. The second patient described by Quick had a daughter with a prothrombin time of 14 seconds, a clearly abnormal result according to this author.

A remarkable feature of all the studies made in families of patients with Factor VII deficiency of the Ackroyd type has been the constant pattern of the abnormal laboratory findings in the heterozygotes. In all reported cases the prothrombin times have been slightly but significantly longer than the normal control. When the Factor VII concentration in the blood of heterozygotes has been assayed, figures ranged from 24% to 60% with one exception—a child of Owren's patient, who had a proconvertin value of 14%.

Factor VII deficiency associated with laboratory evidence of abnormal thromboplastin generation also seems to be inherited in a recessive manner. The most complete family study is that of Hougie's patient by Graham *et alii* (1957). The patient was born of a consanguinous marriage. The father was dead, but the Factor VII concentration in the blood of the mother was 44% of normal. A paternal uncle and aunt both had Factor VII concentrations of 44%. The patient has five children, all with Factor VII concentrations between 21% and 52%. The maternal grandfather and mother of the patient of Hule *et alii* had prothrombin times of 17 seconds (normal control, 13 seconds). The parents of the patient of Greig *et alii* were first cousins, and a sibling had died of a hæmorrhagic tendency shortly after birth.

There have been two cases reported in the literature of mild Factor VII deficiency. It is probable that these patients were heterozygous for the abnormal gene. Koch *et alii* described a patient with a Factor VII concentration of 60%. She suffered from menorrhagia, but had no other symptoms. Wurzel *et alii* (1954) described an individual who had no clinical evidence of abnormal bleeding, but a prothrombin time of 17 seconds (normal controls, 12 to 13 seconds). The abnormality could be found in three generations of this family. The maternal grandmother, the mother and the patient's two sisters had a similar prothrombin time to that of the patient. The mother's brother and the patient's two children were normal. The inheritance pattern suggests that all those with prolonged prothrombin times were heterozygotes for Factor VII deficiency.

The above evidence indicates that more attention should be paid to prothrombin time values a few seconds longer than normal in otherwise healthy subjects. Studies of the families of these individuals may show more than one member heterozygous for Factor VII deficiency. There is no information available concerning the frequency of the heterozygous state in the population. The blood concentration of Factor VII can be assayed with reasonable accuracy, and it would be desirable to know the normal range of concentration in healthy individuals.

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THE SYNDROME OF HYPOPARATHYROIDISM, ADDISION'S DISEASE AND MONILIASIS[1]

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SUMMARY

An eighth example of the rare combination of idiopathic hypoparathyroidism and Addison's disease is reported. A girl, aged 16 years, had suffered epileptic fits since the age of 10. Investigations showed cerebral calcification, with low serum calcium, raised inorganic phosphorus and normal blood urea nitrogen content. She also showed typical clinical and biochemical features of Addison's disease. Treatment with cortisone and large doses of A.T.10 led to improvement in her clinical state and also in her I.Q., electroencephalogram and electrocardiogram. The initial administration of cortisone led to a fall in serum calcium content, and, subsequently, control of the Addison's disease necessitated larger than usual doses of A.T. 10. The clinical course of this and other previously reported cases provides some evidence of an antagonism between the parathyroid and adrenal glands.

IDIOPATHIC HYPOPARATHYROIDISM is a rare condition, less than eighty examples being recorded in the literature. It is therefore a matter of some surprise and interest to find that in at least seven cases, all juveniles, this condition has been accompanied by Addison's disease, itself a rare disorder in the first decade of life. Chronic moniliasis has also been a feature of several of these cases. In three reported instances (Leonard, 1946; Perlmutter *et alii*, 1956; Whitaker *et alii*, 1956) the presence of both hypoparathyroidism and of Addison's disease has been confirmed clinically and pathologically. In four cases (Talbot *et alii*, 1943; Sutphen *et alii*, 1943; Leifer and Hollander, 1953; Papadatos and Klein, 1954) the diagnosis of the combined syndrome was established clinically only, and the patients are either still living or else autopsy was not obtained. There are four further examples recorded in which it seems highly probable that both diseases were present, but conclusive evidence is lacking (Cabot case report, 1954; Hansen, 1954; Craig *et alii*, 1955, Cases 1 and 2). Further details of the above cases are shown in Table I.

The occurrence of these two endocrine diseases in the same individuals raises questions of the possible relationship of the two glands, and the responses to treatment offer some opportunity to study such relationship. It is for these reasons, beyond the interest merely of rarity, that another example is here reported—apparently the first outside the American literature.

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CASE RECORD

A single girl, aged 16 years, was admitted to the Royal Adelaide Hospital on March 1, 1956, with a history of " fits " for the past six years. An electroencephalogram six years before had shown a generalized slow rhythm but no " spike and wave " complexes. She had had about 24 major fits with minor attacks (*petit mal*) every four weeks.

She had weighed eight pounds at birth and was jaundiced. At nine months she had had rubella, at eighteen months whooping cough, measles about a year later, and mumps at six years. At the age of seven years she was referred for treatment of an ulcerated mouth which dated from birth. At 10 years she was admitted to the Adelaide Children's Hospital after a choking episode, and a piece of meat was removed from the larynx. A few months later she had her first fit. At the age of 12 years she had a corneal ulcer in the right eye, which was treated by tarsorrhaphy. When the eye was opened, a squint had developed, which was subsequently corrected by operation. Her teeth had always been friable and had all been removed at the age of 14 years. She had always been short and had never had normal menstruation, though on one occasion there was slight staining. The skin, always dark, had become more so during the past year, and dark spots had appeared on her lips.

She had been a little backward at school but had progressed as far as to train as a typist. Her parents who were unrelated were alive and well. A healthy sister, who was four years older, was of normal growth and development.

TABLE I

Summary of Reported Cases

Author	Sex	Age (Years) at Onset of Parathyroid Disease	Age (Years) at Onset of Addison's Disease	Age (Years) at Onset of Moniliasis	Other Features
Group A: Established clinically and pathologically					
Leonard (1946)	F	3	10½	—	Died at age of 12 years. No parathyroids, atrophic adrenals
Perlmutter *et alii* (1956) ..	F	10	27	—	Died at age of 30 years
Whitaker *et alii* (1956) ..	M	15½	15	2	Died at age of 16 years
Group B: Established clinically but not pathologically					
Talbot *et alii* (1943)	F	4	7	Present	Died at age of 10 years. No autopsy
Sutphen *et alii* (1943) ..	F	5	12	5	Died at age of 12 years. No autopsy
Leifer, Hollander (1953) ..	M	10	20	—	Alive
Papadatos, Klein (1954) ..	M	5½	10	—	Alive
Present case	F	10	15	1	Alive
Group C: Possible examples, not established					
Cabot Case (1954)	F	3	Low sodium and chloride values terminally	2½	Died at age of 3½ years. No parathyroid. Atrophic adrenals.
Craig *et alii* (1955): Case 1 ..	M	Not proven	6	2	Died at age of 6 years. No parathyroids. Atrophic adrenals
Case 2 ..	M	Not proven	9	3	Died at age of 12 years. One small parathyroid. Atrophic adrenals
Hansen (1954)	M	13	"Several years later"	—	No parathyroids. Atrophic adrenals

Her mother's pregnancy had been uneventful, and delivery was normal.

On examination the patient was obviously pigmented over both exposed and non-exposed surfaces. There were several large dark pigmented areas on the inner aspect of her lips. Her tongue and entire buccal mucosa were affected by a chronic monilial infection, which had also spread to her finger nails. The scalp hair was of normal texture; there was very scanty pubic growth but none in the axillæ. Early breast development had occurred. The height was 57 inches and weight was sixty-four and a quarter pounds. The fourth and fifth fingers were not stunted; in fact all her fingers were unduly long. Blood pressure was 90/65 mm. Hg. Chovstek's and Trousseau's signs were both absent. No other significant findings were noted.

On investigation a barium swallow X-ray-examination revealed a congenital stricture just below the suprasternal notch. This was dilated with great symptomatic improvement. X-ray examination of the chest showed a diffuse mottling in the lung fields. This cleared rapidly after dilatation of the œsophagus and was thought to have been an aspiration pneumonitis. X-ray examination of the skull revealed an area of calcification in the right parietal area of the cortex. The electrocardiogram revealed a long *Q-T* interval, (*Q-Tc* 0·55 second).

Serum calcium determination revealed a level of 4·0 milliequivalents per litre (normal 4·5–5·8). The serum content of sodium was 133 milliequivalents per litre, of potassium 3·4 milliequivalents per litre, and of chloride 94 milliequivalents per litre. The low level of serum calcium was confirmed on three other occasions (3·6 milliequivalents per litre). Normal serum sodium, potassium and chloride values were subsequently recorded, though the serum sodium level was again low on occasion. The serum inorganic phosphate content was 5·0 and 5·6 milliequivalents per litre (normal 1·4–2·6) on two separate occasions. The blood urea nitrogen content was 15 milligrammes per 100 millilitres and the serum cholesterol content 215 milligrammes *per centum*. The carbon dioxide combining power was 25 milliequivalents per litre, and the serum alkaline phosphatase content was 12 King-Armstrong units. Renal function as determined by means of urea concentration and clearance tests was normal. The specific gravity of the urine varied between 1·004 and 1·022.

Investigation of adrenal cortical function revealed a 17-ketosteroid excretion of 0·2 milligramm per 24 hours on two occasions (normal 3–10 milligrammes for this age). A Kepler water test done twice revealed only 5·7% and

9·7% excretion (70% or more is normal) and values of "A" of 0·3 and 1·6 respectively (normal "A" exceeds 30). The abnormality in water excretion was subsequently corrected by the administration of 25 milligrammes of cortisone acetate by mouth two hours before the water was given. A glucose tolerance curve revealed a fasting level of 83, and levels 94, 97, 61 and 101 milligrammes *per centum* at 30, 60, 90 and 120 minutes respectively. An insulin tolerance test performed with half the standard dose, *viz.* 1·5 units given intravenously, revealed a fall from 100 milligrammes *per centum* before to 54, 53, 36, 58 and 61 milligrammes *per centum* at 20, 30, 45, 60 and 90 minutes respectively. The patient had a hypoglycæmic attack at 30 minutes. Two random eosinophil counts revealed values of 388 and 330 per cubic millimetre. X-ray examination of the adrenal areas revealed no calcification. The Mantoux reaction was negative with 1 : 1000 tuberculin.

Her basal metabolic rate was —7%, the bone age was normal, and there was no other evidence of tissue calcification. The visual fields were normal, but faint lens opacities were apparent in both eyes.

An electroencephalogram prepared on April 17, 1956 by Dr. H. M. Birch of Parkside Mental Hospital revealed "runs of spike and wave complexes lasting 1·5 sec. as often as 2–3/min. Between these the graph is abnormal with random waves and spikes. The abnormalities were increased by over-breathing". An intelligence test on April 4, 1956, carried out by Dr. A. W. Meadows of the University of Adelaide, indicated a verbal I.Q. of 84, performance I.Q. of 62, and full scale I.Q. of 70 (Wechsler-Bellevue scale). The findings on complete blood examination were normal, while electrophoresis of serum proteins revealed normal values, except for the gamma globulin which was 1·9 grammes *per centum* (normal 0·7–1·0); the serum albumin content was 3·8 grammes *per centum* and the total globulin 3·2 grammes *per centum*.

In summary, the association of epilepsy with cerebral calcification, moniliasis, lowered serum calcium and elevated serum inorganic phosphate with normal renal function established the diagnosis of idiopathic hypoparathyroidism. The combination of pigmentation with insulin hypersensitivity, defective water excretion and low 17-ketosteroid excretion established the diagnosis of Addison's disease. Normal pituitary function was indicated by normal bone age, early breast development, some pubic hair and normal basal metabolic rate.

Course

The patient was placed on 25 milligrammes of cortisone acetate given orally twice a day. There was rapid symptomatic improvement, the pigmentation faded, and the results of her water excretion test improved. Repetition of the electroencephalogram after three weeks revealed definite improvement. The spike and wave complexes were still present, but the other abnormalities were less marked, and there was less sensitivity to hyperventilation. The serum calcium level, however, fell from 3·6 to 2·8 milliequivalents per litre and did not rise when the cortisone was stopped.

At this stage dihydrotachysterol (A.T. 10) was given in a dose of one cubic centimetre per day (with calcium lactate 30 grains daily) and increased after three weeks to two cubic centimetres per day; but after a further three weeks no improvement in serum calcium level had occurred, though there was considerable subjective improvement and the patient's weight increased. The patient's mother reported that she had more initiative and was more lively. When the patient contracted acute bronchitis, cortisone was restarted (12·5 milligrammes twice a day), but no improvement in serum calcium levels occurred for five weeks. Then, following increase of the A.T. 10 (3 cubic centimetres daily), the patients had to be readmitted to hospital on August 28, 1956, in obvious tetany with frequent spasms. The serum calcium content was 2·9 milliequivalents per litre and the serum phosphate content 4·4 milliequivalents per litre. The reason for the tetany in the face of the relatively unchanged levels of serum calcium was not clear. Alkalosis was excluded by determination of carbon dioxide combining power, which was 25 milliequivalents per litre (normal 24–35). The tetany was effectively treated with intravenous administrations of calcium gluconate.

After this admission the patient inadvertently did not receive any cortisone for 10 days. This was discovered through the noticeable darkening in her pigmentation. During that time the serum calcium level had fallen further to 2·5 milliequivalents per litre. Cortisone in dosage of 12·5 milligrammes three times a day was recommenced, followed by increase in A.T. 10 to 6 cubic centimetres per day. The serum calcium level rose rapidly to 3·5 milliequivalents per litre before the increase in A.T. 10; after that the level rose to 3·8 milliequivalents per litre with a serum phosphate content of 3·6 milliequivalents per litre.

At this stage it became apparent that return of the serum calcium level to normal would

require much bigger doses of A.T. 10. The dosage was eventually increased to 15 cubic centimetres per day, with return of serum calcium to normal levels, though the serum inorganic phosphate content still remained slightly elevated at 3·4 milliequivalents per litre. This was associated with further subjective improvement and weight increase. Another electroencephalogram on April 9, 1957, revealed almost complete absence of the spike and wave complexes in the resting record and a much decreased frequency following hyperventilation, the record being more regular than that taken five months earlier when the serum calcium content was 3·6 milliequivalents per litre.

An electrocardiogram now showed a normal *Q-T* interval. Creatinine clearance was now 91 millilitres per minute, compared with 42 millilitres per minute before treatment. X-ray examination of the skull revealed no change in the intracerebral calcification. Another determination of the I.Q. revealed significant improvement of the performance I.Q. (verbal I.Q. 89, perfomance I.Q. 88, and full scale I.Q. 86).

TABLE II

Observation	Values (Milligrammes per Hour) at Intervals (Hours)				
	—3	—2	—1	1	2
Control	20	17	16	19	18
Parathormone given at 0 hours	21	16	12	56	34

The patient's ability to respond to parathormone was demonstrated by measurement of urinary phosphate excretion before and after the intravenous injection of 60 units compared with a control day. The values are shown in Table II.

Her weight was now seventy-four and a half pounds. Her height was unchanged. However, the monilial infection had extended to involve other finger nails. Serum protein determination by electrophoresis showed the gamma globulin level to be 2·1 grammes *per centum*, the beta globulin level 1·1 grammes *per centum* (normal 0·6–1·0), the total globulin 4·0 grammes *per centum* and the serum albumin content 5·4 grammes *per centum*. The result of examination for L.E. cells was negative.

At this time the patient was having, in addition to cortisone and A.T. 10, two grammes of calcium gluconate and three grammes of salt daily. On this regime, while normal levels of calcium were attained, serum sodium and chloride levels were below normal. On May 20, 1957, it was decided to add fluorohydrocortisone acetate 0·125 milligramm on alternate days and to stop the salt. This was followed by normal levels for serum sodium and chloride, and a further weight gain of eight pounds in five weeks. At present the patient is in full-time employment as a typist.

DISCUSSION

It would appear that hypoparathyroidism had been present in this case for at least six years. The clinical features conformed to those described when this condition occurs alone (*Steinberg and Waldron, 1952*; *Robinson et alii*, 1954; de Mowbray *et alii*, 1954). Pseudohypoparathyroidism was excluded by the brisk phosphate diuresis in response to parathormone. Replacement therapy corrected most of these hypoparathyroid abnormalities, as has been frequently described in examples of the isolated disease. In this connection it is interesting to note that, contrary to the experience of Mortell (1946), there was, in this case, a significant improvement in performance I.Q. after therapy. Similar findings have been reported by Camp (1947). Marked improvement also occurred in the electroencephalographic records, as described by Simpson (1952). The date of onset of Addison's disease in this case is difficult to establish. Judged by the increasing pigmentation and by the lack of untoward effects following surgical operations earlier, an estimate of about one year could be made. Here again, the clinical and biochemical features of the hypoadrenocortical state did not differ from those appearing in the isolated disease, and the expected response to replacement therapy occurred. As is usual, the patient made better progress when given a mineralocorticoid (9α fluorohydrocortisone) as well as cortisone.

Perhaps the major interest in the present case, however, centres on the observations made during treatment, which give some indication of the effect of correction of the hypoadrenal state on the hypoparathyroid condition and vice versa. During the initial three weeks' course of 50 milligrammes of cortisone daily there was a fall in serum calcium values. Cortisone therapy was then stopped, but no rise in serum calcium level occurred, despite the administration of two cubic centimetres of A.T. 10 daily. The resumption of cortisone in doses of 25 milligrammes daily was followed five weeks later by gross tetany, requiring readmission of the patient to hospital, although the A.T. 10 had been increased to three cubic centimetres daily.

It was eventually necessary to give 15 cubic centimetres of A.T. 10 daily in order to achieve normal serum calcium levels. This very large dose is similar to that required in the case described by Liefer and Hollander (1953). A similar antagonistic effect of treatment of the hypoadrenalism on the hypoparathyroid state may have occurred in the case reported by Whitaker *et alii* (1956), in which the hypoparathyroidism was not revealed until the Addison's disease had been treated for six months, when serum calcium levels fell sharply and tetany developed. Similarly, Leonard (1946) recorded the development of tetanic convulsions in her patient after the administration of adrenocortical extract. Papadatos and Klein (1954) noted in their case that the maintenance requirements for vitamin *D* varied with cortisone dosage. These observations recall other experiences, such as that of Moehlig and Steinbach (1954), whose patient with post-operative hypoparathyroidism developed tetany when given cortisone for rheumatoid arthritis. Colcher *et alii* (1953) found that the hypocalcæmia in sprue was aggravated by cortisone. Hypercalcæmia in sarcoidosis is diminished by cortisone (Anderson *et alii*, 1954; Mills and McSwiney, 1956; Henneman *et alii*, 1956). The opposite situation, the aggravation of the adrenal deficiency by treatment of the hypoparathyroidism, appears to have occurred also in this compound syndrome. The patient described by Sutphen *et alii* (1943) died a few weeks after institution of adequate vitamin *D* therapy. In the case of Leonard (1946) an episode of general deterioration treated by parathormone injections was swiftly followed by an Addisonian crisis. Leifer and Hollander (1953) record that Addison's disease rapidly became manifest after the vitamin *D* dosage for hypoparathyroidism had been increased to 800,000 units daily. Papadatos and Klein (1954) discovered their patient to be suffering also from Addison's disease only after the hypoparathyroid state had been energetically treated. The mechanism of this postulated antagonism between the parathyroid and adrenal hormones must be purely speculative at present, though a possible site for such action would be the kidney, since it has been shown that cortisone increases urinary calcium excretion in normal subjects (Fischer and Hastrup, 1954; Mills and McSwiney, 1956).

The ætiology of this syndrome is quite unknown. As may be seen from Table I, it occurs mainly in juveniles, with an onset which is usually insidious, probably in infancy. Idiopathic hypoparathyroidism alone has a similar incidence (Steinberg and Waldron, 1952). Addison's disease on the other hand is rarely found in childhood (Welch, 1957). The finding of Addison's disease in nearly 10% of the reported cases of the rare condition of idiopathic hypoparathyroidism strongly suggests that the association is not purely coincidental.

Moniliasis is a remarkably frequent association with idiopathic hypoparathyroidism, being present in 11 out of 56 cases (McLean, 1954). Moniliasis has also been present in four of the eight established cases of the combined syndrome (Table I). It seems reasonable to assume that the moniliasis in the combined (syndrome is primarily related to the hypoparathyroidism. Although it has been suggested that moniliasis might be the cause of the hypoparathyroidism (Sutphen *et alii*, 1943), it seems more probable that the moniliasis is superimposed on the dystrophic changes in the nails and mucosæ. In the present case, however, the moniliasis has persisted and even extended despite biochemical control of the hypoparathyroidism.

A feature of this syndrome which has received little attention is that of associated liver disorder. Whitaker *et alii* (1956) drew attention to the presence, in their case, of changes in the liver of post-hepatitic type. Somewhat similar liver changes were recorded in both the other autopsied cases in this series (Leonard, 1946; Perlmutter *et alii*, 1956). Similar liver findings were reported by Craig *et alii* (1955) in their two cases (Table I). In the present case, there is a definite abnormality in the plasma protein pattern, with elevated total globulin level (4·0 grammes *per centum*, this increase being mainly due to gamma globulin. Apart from the moniliasis (an unlikely cause) there is no evidence of any chronic infection, so that this hyperglobulinæmia may be an indication of liver damage. The possibility of this whole syndrome being the result of virus infection was raised by Whitaker *et alii* (1956), but there is no definite evidence to support this view. The possibility of "lupoid hepatitis" (Mackay *et alii*, 1956) was excluded by the absence of L.E. cells in the present case. However, the striking elevation of gamma globulin in this patient does recall the similar finding in Hashimoto's disease (Doniach and Hudson, 1957), and the recent demonstration of auto-antibodies to normal thyroid gland (Roitt *et alii*, 1956) in this condition. Evidence of auto-antibodies to normal adrenal glands has been reported in two cases of Addison's disease by Anderson *et alii* (1957). The possibility that the elevation of gamma globulin in this patient is associated with antibodies to adrenal and parathyroid glands merits further investigation.

There is evidence of a familial or congenital defect. The patient described by Sutphen *et alii* (1943), whose parents were first cousins, had two siblings who had idiopathic hypoparathyroidism and chronic moniliasis. The girl reported by Talbot *et alii* (1943) had a brother who died of Addison's disease and moniliasis and possibly had hypoparathyroidism also (Craig *et alii*, 1955, Case 1). The patient in Case 2, who was recorded by the same authors to have Addison's disease and moniliasis, and who had only one small parathyroid gland, had a brother who was described by Collins-Williams (1950) as suffering from idiopathic hypoparathyroidism and moniliasis. Warkany (1954) mentions two siblings, one with idiopathic hypoparathyroidism and the other with Addison's disease. Thus nine examples either of the combined syndrome or of one or other of the components have come from four families.

In seven of the eight established cases of the combined syndrome, the hypoparathyroidism preceded the appearance of Addison's disease by many years (Table I). In the apparent exception (Whitaker *et alii*, 1956), while hypoparathyroidism became manifest following intensive treatment for Addison's disease, the patient had had teeth deformities, alopecia and moniliasis for many years; and at autopsy only two months after the diagnosis of hypoparathyroidism, no parathyroid tissue whatever was found. If, as seems probable, the hypoparathyroidism antedates the hypoadrenalism, it is tempting to speculate whether the latter could in some way be induced by the hypoparathyroidism. As discussed earlier, there is some evidence that parathyroid activity is antagonistic to that of the adrenal, and it may be that, in the absence of such activity, adrenal atrophy might be hastened. This, however, is purely speculative, and it should be noted that idiopathic hypoparathyroidism has also been reported with hypothyroidism (Himsworth and Maizels, 1940) and with panhypopituitarism (Rupp and Paschkis, 1953).

Although no conclusions can be drawn as to the cause of this curious syndrome, it does seem possible that it represents a genetically determined failure of development of parathyroid and adrenal glands, leading to clinical hypoparathyroidism with a susceptibility to chronic monilial infection, and eventually to Addison's disease.

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HÆMOLYTIC TRANSFUSION REACTION DUE TO ANTI KIDD[1]

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SUMMARY

Two hæmolytic transfusion reactions occurred in a patient suffering from rheumatoid arthritis and were shown to be caused by the development of an immune anti-Kidd antibody in the patient's serum. The activity of this antibody disappeared when the serum was heated or kept for a short time in a refrigerator, but could be restored by the addition of fresh serum. The antibody was not present in a sample of serum collected from the patient three weeks after the last transfusion.

The Kidd blood group system was first described in 1951 by Allen *et alii*, who found an unusual antibody in the serum of a mother whose sixth infant suffered from hæmolytic disease of the newborn. The authors postulated that the antibody, which they termed anti-Jk^a, reacted with a corresponding red cell antigen, Jk^a, in both the homozygous and heterozygous states. Approximately 75% of English and American people reacted with anti-Jk^a. Subsequently the antithetical antibody, anti-Jk^b, was found by Plaut *et alii* (1953). Further examples of anti-Jk^a have been reported. Mollison (1956) claims to have been the first to demonstrate, using red cells laballed with Cr^{51}, that anti-Jk^a could destroy $Jk(a^+)$ red cells in the circulation.

This paper describes the occurrence of two hæmolytic transfusion reactions in a patient whose red cells lacked the Jk^a antigen but whose serum contained anti-Jk^a. It describes also some of the unusual reactions given by the antibody.

CASE REPORT

A 29-years-old woman who had suffered from rheumatoid arthritis for 15 years was admitted to the orthopædic ward of the Royal Prince Alfred Hospital on July 3, 1955. Her only pregnancy was terminated for therapeutic reasons after 10 weeks' gestation in 1949. She was transfused in 1950 with 500 millilitres of group A Rh-positive blood, and again in 1951 with 1000 millilitres of group A Rh-positive blood. The patient was group A Rh-positive (CDe/cde).

An acrylic head arthroplasty was performed on the right hip joint on July 26, 1955. After the operation some post-operative shock occurred, and 1500 millilitres of group A Rh-positive blood were administered over a period of three hours without any recorded untoward effects. On August 4, 1955, the hæmoglobin value was 6·9 grammes *per centum*, and a transfusion of group A Rh-positive packed cells was started. Thirty minutes after this transfusion commenced, the patient began to shiver and complain of pain in the joints, especially in the right hip joint, and later became nauseated. The transfusion was stopped three hours after its commencement, when 150 millilitres of the packed cells had been given. The patient's temperature rose during the next two hours to 103° F. A specimen of urine at this time was noted to be red in colour, but was not examined chemically. Specimens of urine obtained on the next day did not contain hæmoglobin or red cells.

A further transfusion was commenced on August 7. The same type of reaction occurred, and the transfusion was stopped. Both serum and urine were found to contain hæmoglobin and methæmalbumin. All cross-matching tests had been performed by the polyvidone method of Ward (1953) and were reported compatible. A sample of serum collected on August 3 and kept in the refrigerator was used to check the compatibility of the red cells for the transfusion on August 7, but no incompatibility could be demonstrated. No suppression of urine was noted after either transfusion in August.

INVESTIGATION OF REACTION

The patient's serum, collected on August 7, 1955, was tested against red cells of the donors used for the transfusion on that date, and a fine agglutination was detected by the saline and indirect Coombs tests. This was due to an unusual antibody which agglutinated the red cells of 53 out of 67 (79%) donors taken at random. There was no relationship between this antibody and those of the ABO, MNS, Rh, Lewis, Kell, P, Lutheran or Duffy blood group systems. Subsequently it was shown that the reactions observed were those expected of anti-Kidd, and the authors are indebted for assistance in this identification to Mr. R. T. Simmonds of the Commonwealth Serum Laboratories, Melbourne, and to Dr. Ruth Sanger of the Blood Group Research Unit,

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Lister Institute, London. The antibody could be detected by the saline tube, albumin tube, P.V.P. and indirect Coombs tests.

The nature of the antibody was further investigated with the following results:

1. Activity of the serum was apparently destroyed by heating to 56° C. for 20 minutes or to 61° C. for three minutes. After heating, the antibody failed to agglutinate the appropriate red cells in saline, albumin, P.V.P. or indirect Coombs test. Activity was restored, however, by the addition of fresh group AB serum.

2. Activity of the antibody was destroyed during storage in a refrigerator at 4° C. or in the frozen state. At first the activity could be restored by the addition of fresh serum, but later this proved impossible even with concentrated fresh serum.

3. A sample of blood obtained from the patient three weeks after the last transfusion did not contain demonstrable antibody.

4. The antibody appeared to be in the beta-globulin fraction of the serum. Equal parts of 16% gamma-globulin solution were added to the Coombs (anti human globulin) serum before the indirect Coombs test was performed. This "neutralized" Coombs serum did not react with Rh-positive red cells coated with anti-D antibodies (gamma-globulin), but did react with red cells coasted with the patient's serum. This technique is based on that described by Cutbush *et alii* (1955).

Discussion

It is reasonably certain that the patient experienced intravascular hæmolytic reactions with the last two transfusions. The transfusion administered on the day of operation may also have been followed by destruction of the donor's red cells, in view of the low hæmoglobin value found nine days later. The Kidd blood group system was probably the cause of these reactions, and the patient was possibly immunized by transfusions which she received in 1950 and 1951.

It is of interest that the antibody caused hæmolysis *in vivo*, but was not hæmolytic *in vitro*. In this respect it resembles the Rh antibodies, but differs from the antibodies of the ABO system. The Jk^a antigen of red cells must be a poor antigen, because the corresponding antibody is only rarely found. This patient may have been unusually susceptible to antigens because of the particular disease from which she was suffering, but we can offer no suggestion as to why she should have been immunized by the Jk^a antigen rather than by any of the other antigens present in the donor's red cells.

The reactions of the antibody are similar to those described for the Lewis antibody by Mollison and Cutbush (1955). Both Lewis and Kidd antibodies appear to be associated with the beta-globulin and to be unstable except in the dried state. Some labile component of serum is necessary for the action of these antibodies, but it is unlikely that complement is concerned. Complement is needed to hæmolyse red cells when an antibody has combined with corresponding antigen on the red cell surface, but it is not required for the union of antibody with antigen. When the serum containing anti-Jk^a was heated and then mixed with red cells, there was no evidence, from the indirect Coombs test, of antigen-antibody union. The factor in fresh serum which is necessary for this union has therefore not yet been identified. Stratton (1956) had earlier noted that fresh serum would reactivate anti-Jk^a.

Acknowledgement

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BODY FAT AND BLOOD PRESSURE OF NATIVES IN NEW GUINEA: REFLECTIONS ON ESSENTIAL HYPERTENSION[1]

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SUMMARY

Natives were examined in the highlands (221 males, 169 females), where salt is scarce, and on the coast (146 males, 87 females). Both groups eat little protein and fat. Measurements were made of height, weight, folds of skin and subcutaneous fat, circumference of arm and blood pressure.

Though shorter and lighter than average Europeans, the relative weight of young adult natives was "ideal". Natives were more muscular and less obese than Europeans.

The blood pressure of young adults was the same as for Europeans (125/80 mm. Hg. at 25 years of age). With advancing age there was a decrease in diastolic pressure with an increase in pulse pressure in both sexes; systolic pressure increased in females but was constant in males. At 65 years of age the average for males was 130/70 mm. and for females 140/75 mm. Hg.

The pulse pressure and the rate of its rise with age resembled the findings in Europeans.

There was no apparent relationship between blood pressure and relative body weight or obesity when allowance was made for errors due to arm circumference. Coastal natives, whose appetite for salt is satisfied, did not have a higher blood pressure than the highlanders, who crave salt.

Results are interpreted to mean that natives are physically as bulky as Europeans (allowing for the difference in height); that the influence of sex and aging of elastic arteries affect both races alike; but that natives lack, while Europeans acquire, the complaint of muscular arteries which is sometimes called essential hypertension.

Unlike people in Australia and other civilized countries, natives in New Guinea are believed very rarely to develop any clinical evidence of coronary, cerebral or other types of vascular diseases. This is the general impression of medical practitioners in the Territory, and it conforms with observations made on other native races. Pathological evidence of arterial disease is also rare; so that one pathologist attributed death to coronary disease only once in 600 consecutive autopsies and found atheroma to be generally absent or minimal (Price, 1956).

The reason for this apparent immunity from diseases which are causing increasing havoc among civilized people is not clear. It certainly cannot be attributed solely to a lower expectation of life. Nor is it likely to be due to a genetically determined absolute immunity, as the experience of other native races has been that the incidence rises when Western modes of living are adopted (Bronte-Stewart *et alii*, 1955). One possible cause which has been extensively investigated in recent years is a low intake of dietary fat. Supporters of this theory claim that fat, through its influence on the concentration of cholesterol in the serum, affects the rate of atherogenesis and the liability to death from coronary artery disease (Keys and Anderson, 1954). Other conditions which are said to be associated with an unduly high incidence of arterial disease include high blood pressure and obesity, both of which are commonly believed to be less prevalent in primitive than in civilized communities.

It is generally conceded that hypertensives, by and large, develop more atheroma than normotensives (Tobian, 1955). However, doubts have been expressed whether hypertension *per se* is the causative factor concerned (Davis and Klainer, 1940) and whether hypertension presdisposes to coronary occlusion (Master, 1953; Gordon, 1955). Indeed, knowledge of cardio-vascular disease is full of doubts. Even the truth of the assumption which is basic to many studies in this field, that clinical evidence of coronary occlusion is a reliable indication of atherosclerosis, is uncertain (Morris, 1951). Nor is there unanimity of opinion regarding the relation of obesity to vascular disease. There are numerous reports in support of a positive association (Shepard and Marks, 1955), but Faber and Lund (1949) found no relation between obesity and atheroma in the aorta, and Garn *et alii* (1951) found no evidence of obesity predisposing towards coronary occlusion, at least in young subjects.

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If obesity has a deleterious effect, then it might conceivably operate by way of an elevated blood pressure or serum cholesterol level.

The survey to be described here was undertaken to collect information on some of these factors which may be playing a part in the natives' freedom from cardio-vascular disease. Other studies in New Guinea have shown that the dietary intake of fat is almost negligible, and that the serum cholesterol level is low, does not increase with age and bears no apparent relation to body weight or the amount of body fat (De Wolfe and Whyte, 1958).

SUBJECTS

Subjects selected for this survey were apparently healthy individuals capable of carrying out their usual tasks which inevitably involved, for most of them, a good deal of physical exertion. Natives were examined in the highlands and on the coast.

Highland Natives.—These were living in Chimbu, 5000 to 9000 feet above sea level, in the eastern part of the Central Highlands of New Guinea. They have had little association with civilization since contact was first made in 1933. They are of good physique and powerful muscular development. Their main diseases are respiratory infections, malnutrition, malaria and dysentery (Ivinskis *et alii*, 1956). Hæmoglobin levels exceed the average European figures, and serum proteins are normal in all respects except for a raised concentration of gamma globulin. The daily food intake of adult males provides about 2800 Calories, 30 grammes or less of protein, and little or no fat and salt (Venkatachalam, 1957).

Coastal Natives.—These lived in small villages in the sparsely populated delta area near Kikori and Aird Hills on the southern coast of Papua. They have had longer contact with Europeans and are affected to a greater extent than the Highlanders by malaria, tuberculosis and other diseases. They probably resemble other coastal natives who have been found to have low hæmoglobin levels, normal serum protein concentration and a diet which provides about 1600 Calories daily, 40 grammes of protein and extremely little fat (Hipsley and Clements, 1947). They do not lack salt.

METHODS

Subjects were assembled and waited at least fifteen minutes before being examined. Observations which were made included the determination of height, weight, blood pressure, and circumference of the arm, abdominal palpation, auscultation of the heart, measurement of subcutaneous fat and, in many of them, inspection of the retinal vessels.

Height was measured to the nearest quarter of an inch. Weight was measured in pounds on portable scales, no allowance being made for ordinary wearing apparel, which usually weighed about two pounds. Blood pressure was measured in the sitting position from the right arm by means of an anaeroid type of machine, which was frequently checked against a mercury sphygmomanometer. The cuff was 13 centimetres wide and of the type fitted with metal braces and clip. Pressure was recorded to the nearest even number of millimetres of mercury, the diastolic being taken at the point of disappearance of sound. Systolic and diastolic pressures measured on a second occasion in 31 subjects after a further period of rest or on the following day gave mean values of 122·2 and 79·1 mm. Hg. respectively, which were significantly lower than the initial readings of 124·8 and 82·3 mm. Hg.

An index of arm circumference was provided for each subject by noting the particular metal stay which was clipped in applying the cuff of the sphygmomanometer. Additional measurements with a tape measure in a series of cases allowed of conversation of mean cuff indices to arm circumference.

Subcutaneous fat was measured as the width, to the nearest millimetre, of the double fold of skin and subcutaneous fat which could be pinched up at three sites—two inches to the left of the umbilicus, at the mid-point of the posterior surface of the left upper arm and over the inferior angle of the left scapula. Measurements are valid only for the purpose of making comparisons, as the spring calipers which were used had spherical contacts, and their closing pressure varied with thickness. The sum of measurements made in the three sites was used as an index of subcutaneous fatness. Measurements made on two occasions in 32 subjects showed good agreement, giving a correlation coefficient of 0·961.

Age was estimated from physical appearance and development, from a history of marriage and the number and size of children, and by inviting comparison with other natives. Ages from twenty years onwards were recorded in multiples of five. Estimates made independently by two observers in 30 subjects agreed in 15 instances, differed by five years in 10 and by ten years in 5.

Results were analysed by statistical techniques described by Fisher (1950) and accepted as showing significant differences or relationships only when the probability of these occurring by

TABLE I

Highland Males: Mean Values (Standard Deviations Shown in Parentheses)

Age Group (Years)	Number	Height (Inches)	Weight (Pounds)	Pounds Weight (per Inch of Height)	Fat (millimetres)	Arm Circumference (centimetres)	Blood Pressure (Millimetres of Mercury)	
							Systolic	Diastolic
11–19	24	—	—	—	—	—	126·33 (13·7)	84·42 (9·1)
20–29	50	61·52 (1·93)	122·34 (11·76)	1·979 (0·162)	19·72 (5·14)	24·43	129·92 (10·9)	83·36 (6·0)
30–39	51	61·64 (1·95)	124·08 (12·77)	2·009 (0·162)	18·61 (6·97)	24·94	124·35 (13·0)	83·06 (9·3)
40–49	38	61·10 (2·45)	119·45 (11·93)	1·951 (0·138)	18·89 (4·26)	24·98	120·58 (11·3)	76·63 (6·5)
50–59	33	60·64 (2·31)	118·97 (11·83)	1·961 (0·178)	20·58 (5·70)	24·03	123·70 (12·4)	75·70 (8·2)
60 and above	25	61·45 (1·93)	113·36 (13·30)	1·840 (0·172)	24·28 (9·96)	22·67	128·40 (16·6)	69·84 (8·0)

chance was less than 5%. The suffixes x, xx, xxx have been used here to indicate probabilities of less than 5%, 1% and 0·1% respectively. It has been assumed for the purpose of analysis that the data were normally distributed. Skewness of distribution was apparent with the skinfold thicknesses (though the g_1 and g_2 values for 158 men 20 to 39 years of age were not remarkable), but no obvious advantages were gained by transforming the data in any of a number of different ways which were tried.

RESULTS

Highland Males

Observations were made on 278 men in the Chimbu area, 57 of whom had been in receipt of regular supplements of food. The remaining 221 led ordinary village lives and provided the data summarized in Table I. The average height of adult males was 61·31 inches, with a standard deviation of 2·14 inches. Body weight decreased with age: the average for all adults was 120·53 pounds (standard deviation 12·71). The body weight per inch of height, sometimes called the ponderal index, showed a maximum value of 2·01 in the fourth decade and a decline in the elderly.

The amount of subcutaneous fat, as indicated by the sum of skinfold thicknesses measured at three sites, showed no real change with age (r=0·097). The apparent increase in the mean value with advancing age was accounted for by a small proportion of the older subjects having relatively thick layers of fat, especially on the abdomen. None was really obese as judged by European standards. Average thicknesses of the double folds of skin and fat for the different age of groups varied between 7·5 and 13·3 millimetres on the abdomen, 3·4 and 3·9 millimetres on the arm and 7·1 and 8·2 millimetres on the back. Aging natives showed a tendency to accumulate fat on the abdomen rather than elsewhere; so that the mean value for abdominal fat increased with age, while measurements on the arm showed only small, irregular changes, and average values for the back actually decreased. Measurements made at each of the three sites showed highly significant correlations ($p<0·001$) with each other and with the sum of the three. The correlation coefficients for each of these relationships for 158 adults under the age of 40 years are shown in Table II; they are slightly better than for those who were older.

The circumference of the upper arm depends largely on the bulk of muscle and partly on the thickness of fat, both of which can vary independently. Overall, there was a decrease in arm circumference with age ($r=-0·220^{x}$, n=278). Among the 158 adult males under the age of 40 years there was a highly significant

TABLE II

Correlation Coefficients for Skin-Fold Thicknesses Measured at Three Sites in 158 Men 20 to 35 Years of Age

	Abdomen	Arm	Back
Abdomen	—	—	—
Arm	0·488	—	—
Back	0·561	0·598	—
Total	0·917	0·687	0·796

relationship between arm circumference and the total estimate of bodily subcutaneous fat ($r=+0·320^{xxx}$), but an even better correlation with the body weight per inch of height ($r=+0·647^{xxx}$). This latter ponderal index doubtless reflected changes in the amount of muscle in the body rather than of fat, in this relatively non-obese population.

Whereas the systolic blood pressure, as recorded, showed no real variation, the diastolic pressure decreased with age. Pulse pressure increased, especially over the age of 50 years. These relationships still held when allowance was made for any effect attributable to variations in the circumference of the arm or of fatness ($r=-0·090$ for systolic and $-0·417^{xxx}$ for diastolic pressures). If the corrections for girth of arms as calculated by Pickering *et alii* (1954) are accepted and applied to these observations, then the same general relationships

with age remain, the estimated true systolic pressures being higher and the diastolic lower than as recorded in Table I. Diastolic pressures "corrected" in this way have no precise meaning, even if the published relation between direct and indirect pressures applies to these natives, since the corrections are based on the diastolic pressure recorded at the sudden muffling of the sounds and not on the disappearance of sound as used here.

There was a positive correlation between the circumference of the arm and the systolic pressure ($r=0\cdot194^x$) and the diastolic pressure ($r=0\cdot223^x$), allowance having been made for any possible influence of age and fatness. There

TABLE III

A Comparison between Men (20 to 35 Years of Age) in the Villages and Labourers Who had Received Extra Food for a Year or Longer

Group	Weight (Pounds)	Arm (Centimetres)	Fat (Millimetres)	Blood Pressure (Mm. Hg)	
				Systolic	Diastolic
Villagers (101)	123·22	24·7	19·16	127·11	83·25
Labourers (49) ..	132·53	27·2	23·20	134·78	85·35

was not, however, any significant association between the amount of subcutaneous fat and the systolic ($r=0\cdot109$) or diastolic pressures ($r=0\cdot096$), age and arm circumference again having been taken into account. Similar relationships were found when attention was restricted to adult men under 40 years of age, who, in addition, showed no significant correlations between blood pressure and the ponderal index ($r=-0\cdot106$ for systolic and $-0\cdot032$ for diastolic pressures: variations in circumference of arm and fatness being allowed for).

In contrast to what is found in European populations the incidence of "hypertensive" subjects decreased as age advanced. Eleven of the 125 subjects under the age of 40 years and none of the 96 older subjects in Table I had blood pressures of 140/90 mm. Hg. or higher.

The Effect of Extra Feeding.—Observations were made on 49 men, 20 to 35 years of age, returning from service on coastal plantations. For at least 12 months, and mostly for 18 or 24 months, these men had been labouring and receiving extra rations such as meat, margarine, sugar, salt, wholemeal and rice. Their daily caloric intake was about 3500 Calories (Venkatachalam). Compared with 101 village-fed men of the same age, they were of similar height and nine pounds heavier, with a greater arm circumference and with more subcutaneous fat (Table III). The systolic blood pressure was distinctly higher than for the villagers ($p<0\cdot001$), but the difference in diastolic pressure was not significant. Using the corrections for arm circumference of Pickering *et alii*, assuming that they apply to these subjects and to diastolic pressures recorded at the disappearance of sound, then the corrected pressures were 131·4/77·1 mm. Hg. for the ordinary villagers and 135·9/77·0 mm. Hg. for the labourers. The difference in systolic pressures was then only just significant at the 5% level, assuming that the same variance applied to the corrected as to the observed pressures. It is clear that extra feeding made no real difference to the diastolic blood pressure and that the increase in systolic pressure was due in large part, if not entirely, to the greater thickness of the arm.

Highland Females

Althogether, 169 females of all ages were examined. They were shorter and lighter, but carried more fat than the men (Table IV). The average height of adult women was 57·97 inches with a standard deviation of 2·30 inches. Body weight and the ponderal index decreased with age. Subcutaneous fat also diminished significantly with advancing age, the trend being most noticeable in the scapula region, for which the mean values fell progressively from 16·5 millimetres in the third decade to

TABLE IV

Highland Females: Mean Values (Standard Deviations Shown in Parentheses)

Age Group (Years)	Number	Height (Inches)	Weight (Pounds)	Pounds Weight per Inch of Height)	Fat (millimetres)	Arm Circumference (centimetres)	Blood Pressures (mm. Hg.)	
							Systolic	Diastolic
11–19	24	—	—	—	30·25	21·7	126·42 (11·52)	83·33 (9·35)
20–29	31	58·25 (2·28)	112·77 (13·50)	1·935 (0·214)	40·90	23·5	122·77 (10·27)	79·29 (9·12)
30–39	35	57·85 (1·92)	105·26 (11·46)	1·808 (0·180)	31·88	22·6	130·28 (14·29)	81·94 (9·33)
40–49	34	58·33 (2·01)	101·06 (9·53)	1·730 (0·136)	26·26	22·0	134·76 (18·36)	79·94 (6·53)
50–59	32	57·15 (2·87)	99·59 (8·60)	1·736 (0·173)	27·38	21·6	134·19 (21·12)	74·75 (7·03)
60 and above	13	58·71 (1·78)	98·77 (7·78)	1·682 (0·131)	29·85	21·1	139·23 (20·22)	74·92 (8·86)

TABLE V

The Average Blood Pressures (mm. Hg) of Highland Men and Women, as Recorded, and after Correction for Circumference of Arm (Pickering et alii, 1954)

Age Group	Number	Systolic Pressure (mm. Hg.)		Diastolic Pressure (mm. Hg.)	
		As Recorded	"Corrected"	As Recorded	"Corrected"
20–39 years:					
Male	101	127·11	131·42	83·25	77·10
Female	66	126·76	133·24	80·70	76·03
Significance of Difference ..	—	n.s.	n.s.	n.s.	n.s.
40 years and over:					
Male	96	123·69	128·89	74·54	69·00
Female	79	135·26	143·40	77·01	73·47
Significance of Difference ..	—	$p<0·001$	$p<0·001$	$0·05>p>0·02$	$p<0·001$

8·5 millimetres in those who were 60 years of age or older. Average measurements of abdominal folds varied between 13·3 and 18·0 millimetres at various ages, and folds on the arm between 4·1 and 6·4 millimetres. The circumference of the arm also decreased with advancing years.

Systolic and diastolic pressures diverged as age increased. The systolic pressure showed a significant increase with age ($r=+0·279^{xx}$), while diastolic pressure decreased ($r=-0·273^{xx}$), allowance having been made for variations in the amount of fat and in the circumference of the arm. Pulse pressure increased markedly. When Pickering's corrections were applied to the observed pressures, the divergence was further increased. The proportion of subjects with a systolic pressure of 140 mm.Hg. or more was greater in the older than the younger age groups. The reverse was the case for those with a diastolic pressure of 90 mm.Hg. or more. Subjects with both systolic and diastolic hypertension of this degree numbered 10 among the 90 women under 40 years of age and three among the 79 older ones.

The amount of subcutaneous fat had no noticeable influence on the systolic ($r=-0·054$) or diastolic blood pressure ($r=+0·076$) when allowance had been made for possible effects attributable to age and circumference of arm.

The blood pressure of highland women exceeded that of men as they got older (Table V). This applied to both systolic and diastolic pressures, even when allowance was made for the difference in girth of their arms.

Coastal Males

A summary of the principal observations made among 146 males in the coastal area is given in Table VI. The average height of adults was 63·89 inches with a standard deviation of 2·26 inches. Body weight decreased in the older men, and so did the ponderal index and the circumference of the arm. The total thickness of subcutaneous fat as measured in this study showed no variation with age.

Taking into account any possible influences of age, circumference of arm and degree of obesity, neither systolic nor diastolic pressures showed any significant relationship to age ($r=-0·172$ and $-0·065$ respectively) or to the thickness of subcutaneous fat ($r=-0·013$ and $-0·021$ respectively).

Characteristics of highland and coastal males are compared in Table VII. The two groups were similar in regard to body weight, thickness of subcutaneous fat and circumference of arm, but the coastal natives were taller. Blood pressures were not significantly different, except that the diastolic pressure was higher among highlanders under the age of 40 years. The proportion of subjects with hypertension (blood

TABLE VI

Coastal Males: Mean Values (Standard Deviations Shown in Parentheses)

Age Group (Years)	Number	Height (Inches)	Weight (Pounds)	Pounds Weight per inch of Hight)	Fat (millimetres)	Arm Circumference (centimetres)	Blood Pressure (mm. Hg.)	
							Systolic	Diastolic
10–19	26	—	—	—	—	—	129·92 (9·61)	79·08 (9·52)
20–29	42	64·36 (2·36)	123·60 (13·75)	1·917 (0·178)	20·71	25·1	133·19 (16·69)	77·52 (9·47)
30–39	37	63·92 (2·25)	123·30 (13·48)	1·926 (0·174)	19·97	25·3	128·92 (14·55)	78·97 (7·65)
40–49	24	63·56 (2·85)	120·12 (14·45)	1·888 (0·203)	20·12	24·8	130·00 (12·87)	78·42 (9·40)
50 and above	17	63·15 (2·63)	114·06 (10·95)	1·804 (0·154)	18·88	23·9	124·82 (15·06)	76·12 (8·58)

TABLE VII

A Comparison between Highland and Coastal Males

Age Group	Number	Height (Inches)	Weight (Pounds)	Pounds Weight per inch of Height	Fat (millimetres)	Arm Circumference (centimetres)	Blood Pressure (mm. Hg.)	
							Systolic	Diastolic
20–39 years :								
Highland	101	61·58	123·22	1·994	20·15	24·7	127·11	83·25
Coastal	79	64·15	123·46	1·921	20·37	25·2	131·19	78·20
Difference	—	p<0·001	—	0·01>p>0·001	—	—	—	p<0·001
40 years and over :								
Highland	96	61·03	117·70	1·926	20·88	24·0	123·69	74·54
Coastal	41	63·39	117·61	1·853	19·61	24·5	127·85	77·46
Difference	—	p<0·001	—	0·05>p>0·02	—	—	—	—

pressure of 140/90 mm. Hg. or greater) was similar in the two regional groups and decreased with age.

Coastal Females

Table VIII is a summary of the measurements made in 87 coastal women. The average height of adults was 69·93 inches with a standard deviation of 2·38 inches. Body weight, the ponderal index and subcutaneous fat significantly decreased with advancing age.

Both systolic and diastolic pressure showed a strongly positive correlation with circumference of the arm. When allowance was made for this and for any possible effect due to differences in the amount of subcutaneous fat, then age was found to have no influence on systolic ($r=+0·070$) or diastolic pressure ($r=+0·084$). Likewise, the partial correlation of subcutaneous fatness with diastolic pressure was of no importance ($r=-0·144$), but there was a suggestion of a relationship, an inverse one, with the systolic pressure ($r=-0·254^{x}$).

Comparison of women from the two regions (Table IX) showed that the coastal ones were taller and lighter and had less subcutaneous fat. Systolic pressures were similar. Coastal women had slightly lower diastolic pressures than highland women, but the difference became insignificant when Pickering's corrections for circumference of arm were applied.

DISCUSSION

One of the principal conclusions to be drawn from this survey is that the blood pressure of young adult male natives in New Guinea is not very different from what is found among Europeans. They differ, however, in not rising with age. This is illustrated in Figure I, where the values for the natives are contrasted with those found among Norwegians in the best study of blood pressure in a population which

TABLE VIII

Coastal Females : Mean Values (Standard Deviation Shown in Parentheses)

Age Group (Group)	Number	Height (Inches)	Weight (Pounds)	Pounds Weight per Inch of Height)	Fat (millimetres)	Arm Circumference (centimetres)	Blood Pressure (mm. Hg.)	
							Systolic	Diastolic
10–19	18	—	—	—	29·44	20·5	124·78 (15·20)	72·22 (8·70)
20–29	22	60·77 (2·66)	100·82 (15·12)	1·656 (0·219)	31·54	21·9	124·91 (17·07)	77·18 (11·00)
30–39	31	60·06 (1·91)	97·84 (11·98)	1·627 (0·163)	28·19	22·2	131·61 (16·33)	77·55 (6·21)
40–49	10	58·75 (2·33)	87·80 (13·61)	1·489 (0·194)	21·90	21·6	137·00 (22·07)	76·20 (12·96)
50 and above	6	58·08 (2·49)	79·17 (8·03)	1·363 (0·119)	15·50	18·9	126·33 (19·69)	65·67 (10·98)

TABLE IX

A Comparison between Highland and Coastal Females, 25 to 39 Years of Age

Group	Number	Height (Inches)	Weight (Pounds)	Pounds Weight per Inch of Height	Fat (millimetres)	Arm Circumference (centimetres)	Blood Pressure (mm. Hg.)	
							Systolic	Diastolic
Highland	66	58·04	108·79	1·868	36·12	23·0	126·76	80·70
Coastal	53	60·36	99·08	1·639	29·58	22·1	128·83	77·40
Difference	—	p<0·001	p<0. 001	p<0·001	0·01>p>0·001	—	—	0·05>p>0·02

has been reported to date (Boe *et alii*, 1957). These two principal features mentioned are not common to all native races (Pickering, 1955; Kean and Hammill, 1949; Ordman, 1948). Lower blood pressures, generally about 100/70 mm. Hg. have been found in the Ceylonese, in the Australian aborigines and in primitive Chinese, without any indication of a rise with age. Chinese of higher economic status, applicants for life insurance, show higher pressures, which increase with age, and Chinese living in Canada have blood pressures comparable to the Canadians. On the other hand, reports from India and the Phillipines give pressures of 115/70 mm. Hg., or thereabouts, for young adults, and these increase with age. Similar observations have been made in Japan, where the increase in the incidence of hypertension with

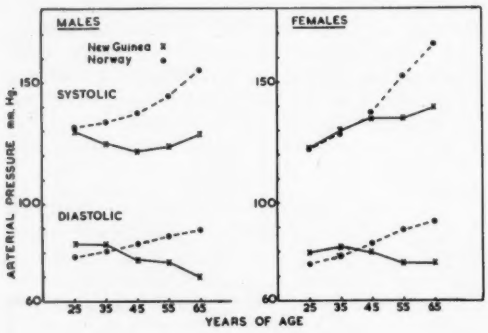

FIGURE I

Blood pressure, as affected by age and sex, in New Guinea (present study), and Norway (Boe *et alii*, 1957).

age is apparently as marked among the poorer classes as among the richer. East African experience resembles the findings in New Guinea—120/80 mm. Hg. in young adulthood and no rise with age. Bantu natives in South Africa, on the other hand, show a marked rise in the incidence of hypertension (diastolic over 90 mm. Hg.) from 8% in the third decade to 40% in the seventh decade. It is of considerable interest that reports of observations among Negroes and the Bantu claim that hypertension is quite prevalent, that atherosclerosis is not uncommon, and yet that clinical evidence of coronary and cerebral vascular disease is rare.

Comparisons of the blood pressures of racial and other groups are fraught with difficulties, as emphasized by Pickering, because of variations in health and nutrition, and because of a general neglect of the influence of arm circumference. In the present study we have been able to take into account some of the variable factors which might be involved, thereby allowing of slightly more precision in interpreting the observations. This is of particular value in comparing the relative weight and obesity of natives with Europeans and in examining the data for the relation of these to blood pressure.

Relative Weight and Obesity

The highland natives are obviously shorter and lighter than Europeans. Averages for young adult males in the third decade are 5 feet 1 inch and 8 stone 10 pounds, and for females 4 feet 10 inches and 8 stone. Personal observations on a group of young Australian adult males gave an average height of 5 feet 10 inches and 11 stone 7 pounds. Comparison is easier on the basis of the ponderal index. For the male native group this was 1·98 pounds weight per inch of height and for the Australians 2·31—that is, respectively, 0·356 and 0·413 kilogrammes per centimetre. This would suggest that the natives are 14% underweight as compared with Australians, but at least part of this difference can be attributed to the fact that the ponderal index is known to vary with height (Boe *et alii*, 1957).

A better index of bodily bulk comparing groups which differ in height is the ratio weight/height^2. This ratio (x1000) is 2·28 kilogrammes/centimetres^2 for the natives and 2·33 for the Australians. The difference here is only 2%, and the indices are practically identical with the mean values found by Boe *et alii* for Norwegians of the same age. The index for native women is greater than for Norwegians, 2·34 as against 2·28. This would suggest that natives are as bulky as Europeans, taking their shorter stature into account.

Further evidence that these natives have a relative body weight which is normal by European standards is obtained by reference to tables of "standard" or "ideal" weights. This has been the commonest method used in surveys for comparing the bodily bulk of different groups of individuals, even though the tables have been compiled from data collected in only one country and may not be strictly applicable to other races with a different physique. If we refer to the tables of the Metropolitan Life Insurance Company, the young male natives are 3% overweight, on the average, and the Australians 7%. The native women are 2% overweight. In contrast to their diet, the weight of natives is "ideal" by American standards.

However, total body weight and indices derived from it do not provide a reliable and sensitive measure of obesity. Within limits,

variations in these indices are likely to reflect changes in the mass of muscle rather than of fat, since muscle is usually the major contributor to total body weight. Extreme variations will undoubtedly indicate obesity or leanness. The best measure of obesity is naturally obtained by calculating the total amount of fat in the body, as can be done from measurements of the specific gravity of the body, or of the total body water, or by using methods involving substances which are selectively absorbed by fat. A simpler method which gives a measure of obesity which correlates well with the total fat content of the body is to measure the folds of skin and subcutaneous fat which can be pinched up at various sites (Keys and Brozek, 1953; Edwards and Whyte). This has been used in the present study and shows quite clearly that natives have less fat than we have found in similar studies among Australians. Thus, the average skinfold thickness of the upper arm of male natives 20 to 30 years of age was 3·8 millimetres and of their Australian counterparts 9·0 millimetres.

Thus, although the young highland natives have the same overall relative weight as Australians, it is obvious that they have proportionately less fat, and therefore more muscle. The fact that these natives excrete in urine at least as much creatinine as Europeans, despite their smaller stature and meat-free diet, may be taken as additional evidence of their muscularity; the average for 20 subjects was 1·23 milligrammes in 24 hours. The coastal male natives who were examined were only 5% below the American standard weight. As they, too, have less fat than Australians, their muscle mass is by no means substandard. With advancing age the indices of relative weight tend to fall, mainly as the result of a decrease in muscle, the fat remaining constant. This is quite different from the general European experience of a rising index and increasing fatness with age.

Relations of Blood Pressure to Obesity

The observations in New Guinea demonstrate a relationship between the blood pressure as measured by sphygmomanometry and the circumference of the upper arm. This is not unexpected, as Ragan and Bordley (1941) have shown that the error involved in the ordinary method for measuring blood pressure, leading to an overestimate, increases with the arm circumference. Nevertheless, it adds confusion to the point at issue, as arm circumference increases with obesity. It also increases with increasing muscularity. However, allowing for the effect of variations in arm circumference and for the influence of age, no significant relationship has been revealed between blood pressure and body fatness or blood pressure and the total body weight relative to height. The range of obesity was, of course, fairly narrow, practically all the subjects falling within ±20% of their standard weight. Labourers who had been eating extra food had more fat and muscle and higher blood pressures than the ordinary village natives. But when estimated corrections are applied for the discrepancy in arm circumference, the difference in diastolic pressure is negligible and the systolic difference is not great. Even if the differences are real ones they could conceivably be due to factors other than body bulk or fatness, such as salt or some other contaminant from civilization.

This lack of association between blood pressure and obesity is contrary to what is generally believed to be the case among Europeans. Pickering has reviewed the evidence and concludes that "a definite correlation between blood pressure and the relation of body weight to height has been found so often as to leave no room for doubt". He goes on, however, to give representative figures and admits the possibility that the differences attributed to variations in weight may be accounted for by the error introduced by varying arm circumference. Fletcher (1954) studied the effects of weight reduction in obese hypertensive women and concluded that the fall in blood pressure was greater than could be attributed to the decrease in girth of arm. Other studies are lacking in which arm circumference is fully taken into account.

Boe *et alii* disagree with the common view, believing that the pure effect of weight on blood pressure is very small when the completely dominating influence of age has been eliminated. They estimate that the systolic pressure increases about 3 mm. Hg. and the diastolic pressure 2 mm. Hg. for every 10 kilograms (one and a half stone) increase in weight; and that the true effect would be less than this if the error due to arm circumference were subtracted. These authors examined more than three quarters of the entire population of Bergen in Norway and have analyzed their observations most meticulously. Unfortunately, as in so many other surveys, arm circumference was not routinely measured. Our own observations in a small series of Australians (De Wolfe *et alii*), which is being extended, confirms their general conclusion. We have found no significant relationship between blood pressure and the skinfold thickness, though there is a

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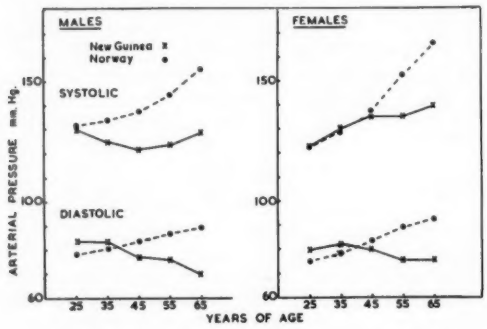

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positive association between blood pressure and the total weight for height, when allowance is made for the effects of age and circumference of arm.

The Influence of Age and Sex on Blood Pressure

Europeans show an increase in blood pressure with age. There is an increase in the mean values and the scatter of systolic, diastolic and pulse pressures, and the magnitude of the rise has been found to be of much the same order in various countries. In all instances the blood pressure of females rises above that of males from middle age onwards.

The male natives in New Guinea show an increase only of pulse pressure with age: diastolic pressure falls, systolic pressure

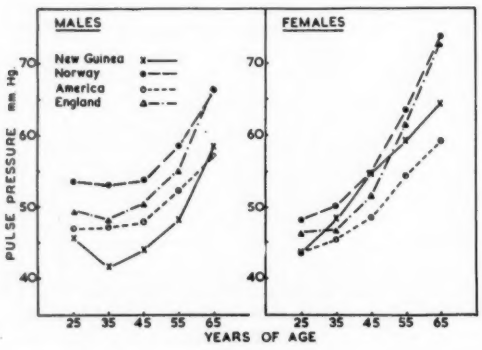

FIGURE II

The increase of pulse pressure with age in different countries. Diastolic pressure was recorded at the disappearance of sound, except for England, where the "muffle-point" was used. Norway: Boe *et alii*, 1957; America: Master *et alii*, 1950; England: Hamilton *et alii*, 1954.

remaining practically constant. Thus there is a change with age, but it is not the same as in Europeans. One point in common is the increasing pulse pressure, and the extent of the increase is similar in the different races, as shown in figure II; even the actual magnitude of these pulse pressures is not grossly dissimilar. These changes with age are probably best explained by a progressive loss of elasticity and increase in volume of the large elastic vessels. Medial degeneration of the type described as "senile ectasia" by Bell and Clawson (1928) could be the pathological basis of the age changes. The main point of interest, however, is that the aging process would seem to proceed at much the same rate in natives as in Europeans. Moreover, the mean blood pressure level is practically the same for natives as it is for young European adults, especially when corrections are made for the difference in arm circumference, as we have been able to do for natives and for Australians. Corrected in this way (which assumes the corrections of Pickering *et alii* to be applicable), the mean pressure for male natives is about 100 mm. Hg., without much change with age. The corresponding figures for our Australian subjects are approximately 105 mm. in the third decade, rising to 130 mm. in the sixth decade.

Our interpretation of these findings is that natives retain throughout life normal physiological control over blood pressure in spite of increasing rigidity of the aorta and large elastic arteries. What part, if any, is played by liver disease and other factors in natives is, of course, uncertain. Europeans, and civilized people in general, suffer the added disadvantage of an age change which reflects on the small, muscular arteries, impairing their ability to preserve the normal, ideal pressure relations which exist in early adulthood. In other word, the essential difference between natives and Europeans in regard to blood pressure is that Europeans develop essential hypertension, which is not a necessary accompaniment of advancing age.

The influence of sex on blood pressure is the same in the natives as in Europeans. Although the diastolic pressure falls with advancing age in native women, it does not fall as far as in the males. Thus, the systolic, diastolic and pulse pressures of native women increase progressively with age above the values found in men. These excesses attributable to sex are comparable to what are found among Europeans, as shown in Figures I and II. The actual blood pressures of native women fall short of European values at all ages to approximately the same extent as the difference between men of the two races. These results can be interpreted as meaning that native women show age changes similar to native men with an additional sex effect similar to what is experienced in Europeans. They differ from civilized women in that they lack essential hypertension.

Essential Hypertension

We agree with the concept put forward by Pickering and his colleagues that essential hypertension is merely a term applied to subjects whose blood pressures fall at the right hand end of a relatively smooth frequency distribution. We also agree that no clear-cut dividing line between normality and hypertension can be distinguished in the scatter of pressures found in any particular age group. However, we

are inclined to believe that there are normal limits for blood pressure, that these correspond to the distribution found among healthy young adults, and that increased latitude should not be conceded to the elderly. In fact, as with atherosclerosis, so with hypertension, it could be claimed that the disorder is so rife in civilized communities, particularly in the upper age groups, that it becomes difficult to find normal controls (Lewis *et alii*, 1956).

More important than to define normality is to discover the ingredient in civilization that causes an increase in blood pressure, moving the pressure distribution progressively upwards with age. The importance of this problem is highlighted by the direct relationship between blood pressure and mortality from some of the major diseases in civilized countries. Any information which leads to prevention of this shift would be valuable. In fact, this problem is exactly analogous to that of atherosclerotic diseases and serum cholesterol where dietary fat and other possible ætiological factors are being so intensively investigated. The serum cholesterol distribution in civilized societies differs from that in native races in being further to the right and rising with age. This has been shown to apply to Australia and New Guinea (De Wolfe and Whyte). As New Guinea becomes civilized, we can expect the distributions of both cholesterol and blood pressure to slide towards the right, especially with increasing age.

The position which any one individual occupies in the distribution corresponding to his age and race is no doubt determined by genetic and other influences. But what are the factors which operate throughout the population to determine where the distribution itself will be centred? Age is often claimed to be a major one, but our own results suggest that it is probably not age itself, but some other factor whose influence is naturally prolonged and perhaps intensified with time. Some individuals apparently react more sensitively than others to this hypertensive influence. The nature of the environmental cause remains obscure. Of diet, obesity, physical exertion and mental stress, which are frequently listed, perhaps diet, in its broadest sense, commands more evidence in its favour than the others. The similarity in blood pressure of the highland and coastal natives, who are comparable in bodily bulk, argues against the ingestion of salt as an important factor. Highlanders are salt-hungry, and the average daily output of sodium in the urine of 20 subjects was found to be 30 milliequivalents. Coastal natives are not desperate for salt and can be assumed to have a greater intake. The excretion of 17-ketosteroids was measured in 10 highland males and was found to be low, even relative to body weight, by European standards—7·4 milligrammes per day. The overall caloric intake of the natives can be assumed to be adequate, in view of their normal relative weight, but not excessive. The diet of both highlanders and coastal natives is deficient in protein and fat, but there is no reported factual evidence to throw suspicion on these elements. Nor have we demonstrated any relation between blood pressure and obesity or muscularity.

The factors which lead to the vascular disturbances which have become incorporated into civilization, and have attracted so much interest in essential hypertension, remain obscure. New Guinea appears to lack them; but its inhabitants lack many things—protein fat, gross obesity, city bustle. However, if the thesis is correct, that the natives have physiologically normal blood pressures and that their body build is normal—which does not apply to all native races—then further study may yield information pertinent to the problems of hypertension and vascular degeneration. Medicine, like painting and sculpture, may find inspiration among the primitives.

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SERUM CHOLESTEROL AND LIPOPROTEINS IN NATIVES OF NEW GUINEA AND AUSTRALIANS[1]

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SUMMARY

Measurements of total serum cholesterol were made in Australians and in natives living in the Central Highlands of New Guinea (Wabag and Chimbu) and on the coast. Dietary fat provides 0% to 5% of the caloric intake in natives and 35% to 40% in Australians.

The total cholesterol level in natives was lower than that of Australians. Males, 20 to 40 years of age, had 223 milligrammes of cholesterol per 100 millilitres of serum in Australia, 144 in Wabag, 130 in Chimbu, and 124 on the coast.

Unlike Australians, the natives showed no upward trend in cholesterol level with age, and the value was lower in males (in Wabag) than in females.

Paper electrophoresis of serum from males 20 to 40 years of age showed that the beta lipoproteins accounted for 78% of the total cholesterol in Australians, 80% in Chimbus and 86% in coastal natives. The percentage tended to increase with the total cholesterol level in Australians but to remain constant, regardless of total level, in the natives.

A group of employed natives who had been eating extra food (18% of calories from fat) for one to two years had the same average total serum cholesterol concentration and lipoprotein distribution as the ordinary village natives.

The natives of New Guinea, like other primitive races, are believed to have a low incidence of coronary artery disease and related vascular disorders. This is in sharp contrast to the high and increasing incidence of atherosclerotic troubles found in Australia and other civilized countries. The precise reasons for this difference are not known with any degree of certainty, but among the many ways in which primitive and civilized communities differ, the diet, and particularly the dietary content of fat, has been prominently suspect. This suspicion arises out of the observations that coronary artery disease is unduly common among people with raised levels of serum cholesterol and that the serum cholesterol level is influenced by the amount, and perhaps the type, of fat in the diet (Keys and Anderson, 1954).

Australians derive 35% to 40% of their total caloric intake from fat, whereas the natives of New Guinea derive 0% to 5% in this way. Obviously, it would be interesting to know how much atheroma is to be found, the incidence of coronary artery disease and the average levels of serum cholesterol among the people of the two countries. There is no reason to doubt the validity of the general impression of medical practitioners that *angina pectoris* and deaths due to myocardial infarction are extremely rare in New Guinea, compared with Australia. One pathologist attributed death to coronary disease only once in 600 consecutive autopsies, the majority of which were carried out on coroner's cases (Price, 1956). It may be of some interest that this sole victim had been in the service of Europeans for many years and had been provided with European food. Atheromatous changes in the aorta and coronary vessels are said to be generally absent, or minimal, among natives of all ages, whereas extensive atheroma is frequently found *post mortem* in Australians.

As for the serum cholesterol, the level is much lower in natives than in Australians as demonstrated by the results we are reporting here. This is not unexpected. Rather more surprising is the fact that the serum cholesterol level is no higher in natives who have been living on a diet containing considerably more fat than the simple village fare. Factors other than serum cholesterol, such as blood pressure and obesity, which are sometimes believed to play a part in atherogenesis, and may contribute

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to the differences we are considering between primitive and civilized communities, have been discussed elsewhere (Whyte, 1958).

SUBJECTS

Wabag Natives

The Wabag subdistrict of the Central Highlands of New Guinea extends westwards from Mt. Hagen and Mt. Giluwe. It is a rugged mountainous area, which was first patrolled in 1935. In 1946 a Government station was established at a height of 6300 feet. Elkin (1953) has described some of the native customs. The natives are stockily built, muscular people, whose average hæmoglobin level is considerably higher than that of Australians (Walsh, 1957). They cultivate gardens, keep pigs and hunt animals and birds. Their diet is composed almost exclusively of carbohydrate, the sweet potato being the principal item of food. Very little protein is eaten and extremely little fat. In many respects the natives, their mode of life and their diet are thought to resemble the Chimbus.

Chimbu Natives

Chimbu is in the Eastern part of the Central Highlands of New Guinea. It is a mountainous region, 5000 to 9000 feet above sea level, and is the most densely populated area in New Guinea. There has not been a great deal of contact with civilization since Europeans first entered the district in 1933. The natives are of good physique and powerful muscular development. Respiratory tract infections are their commonest complaints, but they also suffer from malnutrition, malaria, dysentery and other diseases (Ivinskis *et alii*, 1956). Hæmoglobin levels are higher than the average European figures, and the plasma protein concentration of adult natives is normal, except for a raised content of gamma globulin (Venkatachalam, 1957). Sweet potato is the staple item of their diet, but they also eat taro, yam, banana, maize, beans, nuts, sugar cane, and occasionally insects, animals, snakes or fish. Pigs are eaten only rarely on festive occasions. It has been estimated that the average daily caloric intake of adult males is about 2800 Calories, to which the contribution from fat is so small as to be negligible (Venkatachalam). Natives employed by Europeans are given supplementary rations of food, including meat and margarine, so that their total caloric intake is increased to 3500 Calories per day, 18% of which are derived from fat (Venkatachalam). A number of these was examined.

Coastal Natives

Coastal natives were examined in the villages of the sparsely populated delta area near Kikori and Aird Hills on the southern coast of Papua. These natives have had more extensive contact with civilization, but observations were restricted to those who were living an ordinary village life. Natives in this region are taller and thinner than those in the Highlands and are affected to a greater extent by skin diseases, malaria and tuberculosis. Hæmoglobin levels are probably low by European standards, while the total serum protein concentration is normal. Their average daily intake is probably about 1600 Calories, less than 5% being derived from fat (Hipsley and Clements, 1947). Sago is the staple food. Major differences from the diet of Highlanders would include coconuts, sea foods and salt.

Australians

Samples of blood were collected from blood donors, hospital staff and a random selection of working men in an industrial organization (de Wolfe *et alii*, 1958). The average daily intake of a group of adult men included in this series was 3100 Calories, 38% being derived from fat.

METHODS

Total cholesterol was measured in duplicate samples of each specimen of plasma or serum by the method of Abell *et alii* (1952). The standard error of the method was 1·6 milligrammes per 100 millilitres of serum, derived from the expression $\sigma_e = \sqrt{\frac{\varepsilon d^2}{2n}}$.

Lipoprotein distribution was determined by measuring cholesterol in the alpha and beta fractions separated by paper electrophoresis (Langan *et alii*, 1955). The proceedure was duplicated on each sample of serum. The differences between duplicate estimations of the percentage of cholesterol carried in the beta fraction showed a standard error of 3·3%.

Most of the samples of blood from natives in New Guinea were collected into small bottles containing measured amounts of dry sodium citrate, streptomycin and penicillin.* Samples were sent by air to reach Sydney within three to seven days. They were immediately examined by the staff of the Red Cross Blood Transfusion Centre, who then separated off the plasma, kept it frozen and subsequently made

* By Professor N. W. G. Macintosh and Mr. B. Lockett, members of a Nuffield Expedition, in Wabag, and by Dr. V. Ivinskis in Chimbu.

TABLE I

The Cholesterol Concentration (Milligrammes per 100 Millilitres of Serum) at Different Ages in Australia and New Guinea Shown as the Mean and Standard Error with the Number of Subjects for Each Group in Parentheses

Group	Age (Years)				
	10–19	20–29	30–39	40–49	50–59
Males					
Australians	—	207±8 (35)	230±5 (75)	238±6 (64)	239±6 (52)
New Guinea Natives					
Wabag	145±8 (6)	143±4 (25)	144±5 (21)	141±3 (21)	137±8 (12)
Chimbu	124±12 (13)	134±7 (22)	107±9 (10)	109±9 (10)	—
Chimbu	Village-fed	137±5 (25)[1]			
Chimbu	Extra feeding	122±6 (19)[1]		—	—
Coast	—	124±6 (23)[1]		—	—
Females					
Australians	193±14 (8)	197±12 (11)	206±8 (9)	220±10 (10)	225±11 (11)
New Guinea Natives					
Wabag	160±5 (17)	155±12 (9)	—	—	—
Chimbu	120±12 (7)	129±8 (20)	119±13 (10)	93±8 (10)	122±21 (5)

[1] Fresh samples of serum.

the samples available to us. Our measurements of total cholesterol content were made up to two years later.

More recently, samples were collected by one of us, from active and apparently healthy men, 20 to 40 years of age, in the Chimbu area of the Highlands and in the Kikori-Aird Hills coastal area. Blood was allowed to clot and then the serum was flown to Sydney in small bottles containing powdered streptomycin and penicillin. Measurements of total cholesterol content and of lipoprotein distribution were made as soon after arrival as was practicable, six to nineteen days after collection. The serum was kept refrigerated but not frozen for as much of the time as possible. The lipoprotein distribution in serum from apparently healthy Australian men, 20 to 39 years of age, was determined after subjecting the samples to antibiotics, agitation and periods of refrigeration and warmth comparable to the treatment received by the samples sent from New Guinea.

Experiments were designed to test the possible influence of anticoagulants, antibiotics, hæmolysis and the delay, agitation and variation in temperature such as were experienced by some of our samples during transportation. These factors, in the degree to which they were judged to be operative, had no significant effect on the level of cholesterol or on the relative proportions of cholesterol which could be recovered from the alpha and beta fractions of the lipoproteins.

RESULTS

The average cholesterol levels found at different ages and in different groups are given in Table I. Most of the figures relating to

TABLE II

Average Values for the Serum Cholesterol, Beta Lipo-protein (with Standard Errors), Blood Pressure and Indices of Bodily Bulk and Fat (Sum of Skinfold Thicknesses at Three Sites) in Males, 20 to 39 Years of Age, in Australia and New Guinea

Group	Serum Chloresterol (milligrammes per 100 Millilitres)	Beta Lipoprotein (% total Cholesterol)	Weight/Height (pounds per inch)	Skinfolds (millimetres)	Arm Circumference (centimetres)	Blood Pressure mm. Hg.	
						Systolic	Diastolic
Australians (20)	247·6	78·1±2·40	2·358	37·9	28·0	132·6	82·4
New Guinea Natives Chimbu—village fed (25)	136·8	80·2±1·46	1·962	20·9	24·8	119·9	80·8
Chimbu—with extra feeding (19) ..	122·2	79·6±1·93	2·120	24·1	27·9	132·9	85·6
Coast (23)	123·6	85·6±1·40	1·956	20·4	25·4	125·5	75·7

New Guinea are from measurements made on the stored samples of plasma. The cholesterol and beta lipoprotein content of the more recently collected, fresher samples of serum are shown in Table II together with other observations made in the same subjects. There was no significant difference between the cholesterol levels of fresh and old samples of blood taken from subjects of similar ages.

Regional Differences

The serum cholesterol levels of natives in New Guinea were obviously lower than those of Australians. This is illustrated in Figures I and III.

Within New Guinea, higher levels were found in Wabag than in Chimbu or on the coast. Thus, the average values (in milligrammes per 100 millilitres of serum) for males, 20 to 40 years of age, were 144 in Wabag, 130 in Chimbu and 124 on the Coast. Coastal natives had much the same caloric intakes as the Chimbus, and in each case less than 5% was derived from fat. These two groups are similar in regard to body weight and thickness of subcutaneous fat, but the

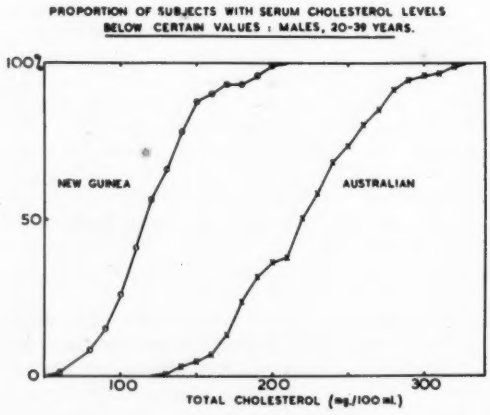

FIGURE I

The distribution of serum cholesterol levels among 76 Chimbu and 130 Australian men, 20 to 39 years of age, shown as the percentage of subjects with levels below certain values.

coastal natives are taller, are affected to a greater extent by malaria and other diseases, and have lower hæmoglobin levels. Nevertheless, the difference in serum cholesterol levels in these two areas was insignificant. Rather more surprising was the finding of a distinct difference between the two Highland groups ($P<0{\cdot}01$ for males 20 to 39 years). The natives of Chimbu and of Wabag are similar in many ways, but it is possible that more fat is consumed in Wabag, as pigs are said to be eaten more regularly than in Chimbu (Healey, 1956).

The beta lipoprotein content of serum was greater in Australian men (193 milligrammes of cholesterol per 100 millilitres) than in natives (about 105 milligrammes per 100 millilitres).

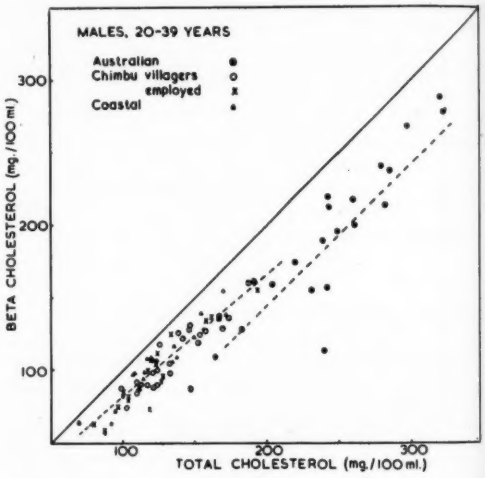

FIGURE II

The relation between the amount of cholesterol in the beta-lipoprotein fraction and the total serum cholesterol level.

However, the same average percentage of cholesterol was found in the beta fraction in Australians (78·1%) as in Chimbu villagers (80·2%), but this was significantly less than in coastal natives (85·6%) The generally close correlation between total cholesterol and the cholesterol incorporated in the beta lipoprotein fraction is shown in Figure II.

The linear regression for the Australian observations was y(cholesterol in beta lipoprotein, milligrammes per 100 millilitres)$=1{\cdot}08\times$(total cholesterol)$-71{\cdot}18$. There was a significant correlation between the total cholesterol level and the percentage present as beta lipoprotein ($r=+0{\cdot}471$; $P<0{\cdot}05$).

The relation of beta cholesterol to total serum cholesterol was substantially the same in coastal and in Chimbu natives; the linear regression was y(beta cholesterol, milligrammes per 100 millilitres)$=0{\cdot}84\times$(total cholesterol)$-2{\cdot}60$. There was no significant correlation between the total cholesterol level and the percentage present in the beta fraction ($r=+0.072$).

Effect of Age

The average serum cholesterol level increased with age in Australian males and females. In Wabag males the cholesterol concentration appeared to remain constant at all ages (Figure III). Other groups had smaller numbers of subjects but showed no upward trend: in fact there was a suggestion of a decrease in cholesterol with age in Chimbu males.

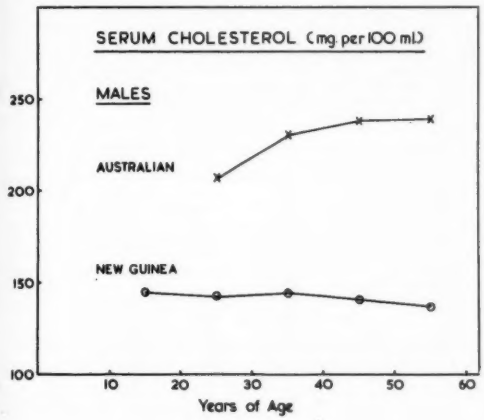

FIGURE III

The serum cholesterol concentration at different ages in Australians and natives of Wabag.

Sex Differences

Australian males, had, on the average, higher cholesterol levels than Australian females. The reverse was true for Wabag natives aged 10 to 30 years ($0{\cdot}02 > p > 0{\cdot}01$). No significant difference was demonstrated in the Chimbus.

Effect of Extra Feeding

Chimbu labourers had a greater caloric intake than the ordinary Chimbu Villagers, and their dietary fat provided 18% as opposed to something less than 5% of the total calories. The labourers had been working on coastal plantations for at least 12 months on rations which included tinned meat and margarine. The labourers were heavier and fatter than the villagers and had a lower average serum cholesterol concentration, but the difference did not quite reach a significant level ($0{\cdot}1 > p > 0{\cdot}05$). The beta lipoprotein concentration was the same in both groups (Table II).

Relation to Obesity

Obesity in the European sense was not observed among the natives in New Guinea, nor were obese individuals included among the Australian men whose blood was examined for lipoprotein distribution. Nevertheless, the Australians were heavier in relation to their height and had nearly twice the thickness of subcutaneous fat (Table II). In no group—Australian, Chimbu or coastal—was there demonstrated any significant correlation between either bodily bulk (weight per inch of height) or bodily fat (the sum of skinfold thicknesses in three sites) and the total cholesterol level or the percentage or the absolute concentration of cholesterol present as beta lipoprotein. Only small numbers of subjects were examined: similar negative results were found when the native groups were combined. Observations made in a larger group of Australians will be reported later (de Wolfe *et alii*, 1958).

Relation to Blood Pressure

No significant relationships were demonstrated between the serum lipid measurements and the systolic and diastolic blood pressures of the small groups of subjects on whom these observations were made. Average findings are shown in Table II. There were no striking differences in blood pressure, especially when allowance was made for variations in the average girth of arm (Pickering *et alii*, 1954).

DISCUSSION

The finding of a low average serum cholesterol level in the blood of natives of New Guinea is not at all unexpected in view of what has been found among other native races. The results are of the same order as those reported by Mann *et alii* (1955*a*) for Nigerians and by Gopalan and Ramanathan (1956) for low-class Indians. Dietary fat provides 8% to 15% of the caloric intake of Nigerians, 4% to 13% for the Indians and less than 5% for the natives of New Guinea. It is difficult to conceive of any group of people naturally consuming less fat than the native subjects of the present investigation. Thus, the average serum(cholesterol level of Chimbu natives, about 130 milligrammes per 100 millilitres, probably represents the lower limit for racial cholesterol values in so far as they are influenced by fat in the diet. The relative position of New Guinea in the dietary fat-serum cholesterol pattern, together with some of the other countries and racial groups for which observations have been reported, is shown in Figure IV.

The diet in New Guinea lacks protein as well as fat. Variations in protein intake have been shown to have no influence on serum cholesterol levels in subjects accustomed to American standards of nutrition (Keys and

Anderson, 1957). Habitual protein deficiency, however, such as exists in New Guinea and among most races whose diet contains little fat, might well affect cholesterol metabolism and the response to feeding extra fat. Liver disease in protein-deficient individuals could also play a part, though Walker and Arvidsson (1954) found no relation between serum cholesterol level and biochemical evidence of hepatic dysfunction in South African Bantus.

Even though the exact reasons for low cholesterol values in native races are uncertain, this does not detract from the popular theory which attributes to cholesterol a prominent

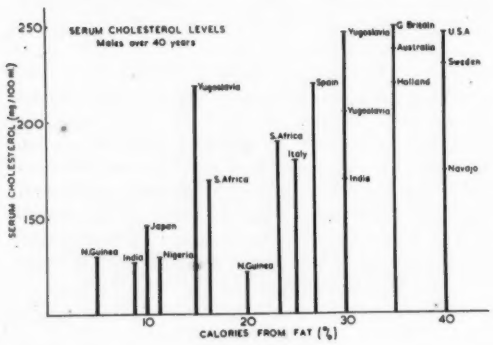

FIGURE IV

Some of the published figures for serum cholesterol levels in males, mostly over the age of 40 years, in different countries in relation to the dietary intake of fat. Where there are figures available for different groups in a community, subsisting on different amounts or types of fat, the country is represented more than once, for example, New Guinea.

role in atherogenesis and in the ætiology of coronary heart disease. The incidence of these disease processes in New Guinea and in Australia appears to differ, at least in direction, in much the same way as the average serum cholesterol levels. But if cholesterol plays any substantial role in atherogenesis, then it is probably due to the size and unstable lipid burden of the beta lipoprotein particles. The concentration of these is very much lower in New Guinea natives (105 milligrammes per 100 millilitres) than in Australians (193 milligrammes per 100 millilitres), though in each case they account for much the same proportion, about 80%, of the total cholesterol. There is no obvious explanation for the slightly higher percentage found in the coastal natives, and it would be purely speculative to attribute this to the nature of the fat they eat, such as the highly saturated coconut oil. Uncontrolled technical difficulties due to climate and other factors connected with the transport of samples by canoe may have played a part. For similar reasons it is difficult to compare results obtained by different methods in various parts of the world. However, Mann and his colleagues, using the ultracentrifige, found that Nigerians and Americans had the same relative amounts of beta lipoproteins (Sf 12–20 *plus* Sf 20–100) in spite of a marked difference in total cholesterol levels. On the other hand, Bronte-Stewart *et alii* (1955) found by paper electrophoresis that Bantu natives had 72% of their total cholesterol (166 milligrammes per 100 millilitres) in the beta fraction, Cape-coloured natives eating more fat had 77% (of 204 milligrammes per 100 millilitres) and South African Europeans had 83% (of 234 milligrammes per 100 millilitres).

New Guinea natives, at least those in the Highlands, differ from Australians not only in the levels of total cholesterol and beta cholesterol, but also in the manner in which increments in the serum cholesterol content are distributed between the alpha and beta lipoproteins. Observations in the Australian men, 20 to 39 years of age, accord with what has been found in European normal and atherosclerotic men—namely, that with the higher levels of serum cholesterol the beta fraction is disproportionately increased while the alpha fraction actually decreases (Barr *et alii*, 1951; Oliver and Boyd, 1955). The higher the cholesterol level, the greater is the percentage of it carried as beta lipoprotein. On the other hand, serum of male natives contains a relatively constant percentage of the total cholesterol in the beta fraction, regardless of the actual total concentration of cholesterol. Thus, increased amounts of cholesterol are accounted for by proportionate increases in both alpha and beta lipoproteins. The significance of this finding is uncertain, and further observations are required. But if the relationships found here are true, then it would mean that natives have more beta lipoprotein and less alpha lipoprotein than Australians when the total cholesterol level is below 250 to 300 milligrammes per 100 millilitres of serum. Taking the New Guinea and Australian results as a whole, the beta cholesterol would account for 81% of the total cholesterol (see Figure II).

The observations among natives in receipt of extra food suggest that an increased consumption of fat was without effect on the serum cholesterol level. There was no evidence of its having risen, in spite of an increase in dietary fat intake from virtually none to 70 grammes daily (0% and 18% respectively, of the total calories)

and in spite of a positive caloric balance as suggested by the increased body weight and subcutaneous fat. We have no exact knowledge of the constitution of the fats consumed, but it is reasonable to believe that they were predominantly saturated. Increased physical activity may have played some part in maintaining the low level of cholesterol (Mann *et alii*, 1955*b*), but habituation to little or no fat in the diet, liver disease or the presence of some cholesterol-suppressing item of diet may have been concerned. Feeding saturated fat for short periods has been shown to cause the serum cholesterol to rise in many groups of subjects, including South African Bantus with low initial serum levels and accustomed to a

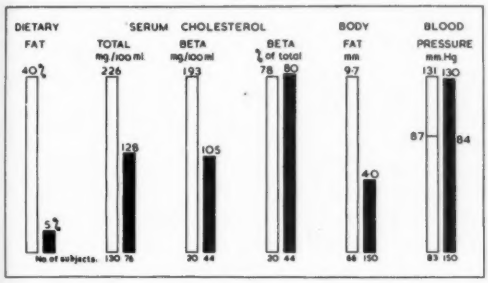

FIGURE V

A comparison of some of the factors accused of influencing atherogenesis in Australians (open columns) and in New Guinea natives (solid columns). Most of the data refer to males, 20–39 years of age. Body fat is given as the skinfoldthickness of the arm (millimetres). The numbers of subjects concerned are given at the foot of each column.

diet in which 17% of the calories came from fat (Bronte-Stewart *et alii*, 1956). Keys *et alii*, (1957) demonstrated a similar effect in Japanese subjects whose basal fat intake provided 10% of their total calories. Butter, margarine from animal fat and margarine from vegetable fat all caused the serum cholesterol to rise, the magnitude of the rise being approximately the same as for Minnesotans. The fats were fed for only 11 days, and the authors recognized the possibility that long-term effects might not be the same. Our subjects had been having supplementary food for at least twelve months, and mostly for eighteen months or two years. We hope to conduct some more acute experiments and to make serial observations.

However, important dietary fat may be in the genesis of atherosclerosis and coronary heart disease, other factors, no doubt, play a part. Some of these, such as heredity, sex and specific metabolic disorders may exert their influence, at least in part, by way of their effect on the serum cholesterol. Some may encourage the formation of thrombi. Others, such as hypertension, may act mechanically. All these factors, and others which are possibly of importance, such as physical activity, obesity and the stress and strain of modern civilized living, are likely to contribute to the difference in the incidence of these diseases in Australia and New Guinea. Some of the relevant observations reported here and elsewhere (Whyte, 1957) are illustrated in Figure V.

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ACUTE HÆMORRHAGIC LEUCO–ENCEPHALITIS[1]

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SUMMARY

Twenty-three cases of acute hæmorrhagic leuco-encephalitis have been described in the literature under this title. The clinical and pathological features of these cases have been reviewed.

The condition is characterized by the sudden onset of speech disturbances, motor paralyses and urinary incontinence. Rapid progression to coma and death has occurred in all cases.

The illness generally follows a respiratory infection after a short latent interval. It may also arise spontaneously, or follow the administration of certain drugs or chemical agents.

At autopsy, the lesions in the cerebral hemispheres are almost entirely confined to the white matter. Elsewhere in the central nervous system, this predilection is not obvious. The lesions consist of areas of hæmorrhage, perivascular exudate, fibrinoid degeneration of blood vessels, especially of small veins, together with focal and perivascular areas of demyelination and microglial proliferation.

Present evidence suggests that the condition may result from hypersensitivity—a cerebral Arthus phenomenon, and that this state may be induced by different allergens. Auto-sensitization may be involved.

The relationship of this condition to various types of " hæmorrhagic encephalitis ", and to acute disseminated encephalo-myelitis is discussed. It would appear that what is known as acute hæmorrhagic leuco-encephalitis in Australia, the United Kingdom and certain parts of Europe, is called acute necrotizing hæmorrhagic encephalopathy in the United States.

The use of cerebral biopsy in an attempt to obtain a definitive diagnosis during life is advocated.

The early administration of cortisone in the diagnosed case is suggested.

HURST, in 1941, described two cases, and possibly a third, of an " acute cerebral condition, virtually localized as far as the cerebrum was concerned to the white matter, and developing more or less abruptly in apparently normal individuals. Perivascular necroses, perivascular and focal demyelination, hæmorrhages, œdema and cellular infiltration were the chief components of the pathological picture ". He regarded the condition as a previously undefined entity and gave to it the name acute hæmorrhagic leuco-encephalitis.

In 1954, Crawford reviewed the pathological features of seven reported cases, and added three more of his own. One of the objects of his communication was to draw the attention of general pathologists to the existence of the condition, for he considered that his study of the small group of cases strengthened Hurst's contention that acute hæmorrhagic leuco-encephalitis (AHL) was indeed a specific disease entity distinct from other forms of encephalitis.

Up to the present time, a total of 23 cases have been described in the literature under this title. It is the purpose of this paper to consider the clinical features presented by these cases, in order to determine whether or not a recognizable pattern emerges in the natural history of the disease. The relationship of this condition to other disorders of the central nervous system will be mentioned.

[1] Based on a paper presented before the Neuropathological Society of South Australia on April 4, 1957.

[2] John Barker Research Fellow in Medicine, University of Adelaide.

INCIDENCE

The condition would appear to be extremely rare. Nevertheless, case reports have appeared with increasing frequency over the last few years, and it is probable that many more cases will be detected as the condition becomes more generally recognized. It is interesting to note that fully three-quarters of the cases published so far have originated in either the United Kingdom or Australia, and it is possible, if not probable, that examples of this condition have been described in the United States and elsewhere under the title of acute necrotizing hæmorrhagic encephalopathy or under such other titles as brain " purpura " or " hæmorrhagic encephalitis ".

SEX AND AGE INCIDENCE

The distribution of the cases between the sexes is almost equal. Males have been affected in 12 instances and females in 11 (Figure I).

The age and sex distribution is shown in Figure I. Early reports favoured a male preponderance and a dominating incidence in the third and fourth decades, but this does not seem at all definite, and both sexes are affected to roughly the same extent.

The youngest case was that of a male child of two years and ten months (Crawford, 1954—case 2); the eldest, that of a man of 51 years (Greenfield, 1950). The average age was 29·6 years.

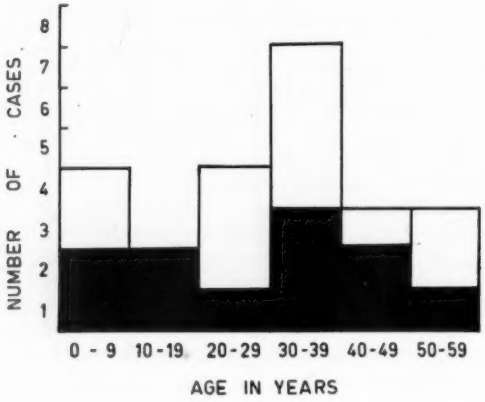

FIGURE I

The age and sex distribution of the patients in the 23 reported cases of acute hæmorrhagic leuco-encephalitis. Females are represented by blocked areas, and males by unblocked areas

THE CLINICAL FEATURES

The clinical picture to be presented has been drawn from a study of only 19 of the 23 reported cases, as few clinical details were given in three of the remaining four cases (Moosy *et alii* (1954)—2 cases, the second of which is evidently the case described by Wolf and Cowen (1946); amd Symmers (1956)—case in abstract). The second case described by Symmers (1956), associated with sensitivity to oxophenarsine, has been omitted from consideration in this respect, on the grounds that the coincident involvement of the central nervous system with the lesions of thrombotic microangiopathy (thrombotic thrombocytopenic purpura) renders analysis of the effect of either disease alone extremely difficult.

In outline, it is possible to divide the clinical picture into three phases (Figure II). There is usually a prodromal period suggestive of respiratory tract involvement. This is followed by an almost, or even completely, asymptomatic interval phase. The final stage commences with the sudden onset of symptoms and signs referable to the central nervous system and terminates in death.

The interval phase may, however, be absent, in which event the prodromal period merges directly into the neurological phase. Occasionally, the neurological stage presents without any preceding ill-health whatsoever.

The duration of each of these periods in the individual cases studied can be seen in Table I.

The Prodromal Phase

The prodromal phase (present in 13 instances) usually lasts between two and five days, but may continue for a fortnight.

The symptoms are those suggestive of respiratory tract involvement with coryza, sore throat, hoarseness and cough. The latter may or may not be productive. Occasionally chest pain of pleuritic type may be present. These symptoms, usually of moderate severity, are frequently accompanied by such evidence of constitutional disturbance as fever, malaise, headache and vomiting.

On physical examination, the signs of an upper respiratory tract infection, of acute bronchitis or of bronchopneumonic consolidation may be found. In those cases in which radiological examination of the chest has been carried out at this stage of the illness, the findings have been non-specific.

The intensity of the symptomatology generally reaches its height early in the illness and then subsides gradually over the next few days, until the next phase is reached.

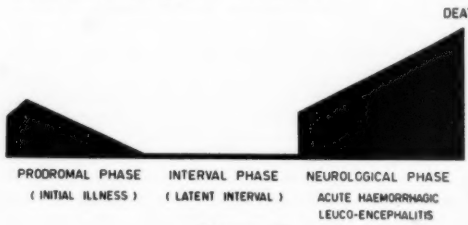

FIGURE II

The clinical picture is represented in three phases

The Interval Phase

This phase, when present, once again lasts only a few days. The patient may be completely symptom-free during this period or may complain only of vague, almost indescribable ill-health. However, it will be seen (Table I) that a clear-cut latent interval was present in only six instances. Nevertheless, a short period of amelioration of symptoms was present in several of the remaining cases.

The Neurological Phase

The neurological phase is characterized by the sudden onset of encephalitic symptoms and signs and in all reported cases has terminated in death. It has been arbitrarily divided into two periods—a precomatose period and a period of coma. It is to be emphasized, however, that this has been done merely to facilitate discussion, for in fact there is no clear delineation between these two periods. The approximate duration of these periods in shown in Table I, where it can be seen that on an average, the time spent in coma (3·3 days) was almost twice that spent in the precomatose phase (1·8 days).

The initial symptoms of this phase are usually those of the constitutional disturbances associated with a moderate or even marked pyrexia, together with severe headache, vomiting and mental irritability. Not infrequently, however, drowsiness and mental confusion are present. Rarely, sudden collapse is the presenting feature. The progress of the condition is rapid, and the clinical picture is soon dominated by the development of such encephalitic manifestations as speech difficulties, sensory and motor disturbances and urinary incontinence.

Disorders of speech generally appear at an early stage in the precomatose period. They were present in seven of the 19 cases described, and varied in degree from an apparent mild nominal aphasia to complete mutism. The dysfunction would appear to be generally cortical in origin.

Motor and sensory disturbances are a prominent feature of the condition. Progressive motor disorders appear early in the neurological phase and generally dominate the clinical picture until death. The manifestations are of considerable variety, but generally present as either a spastic hemiplegia, quadriplegia or paraplegia. Not infrequently the onset of paralysis is preceded by sensory disturbances of cortical type. Involuntary movements are not uncommon, and epileptiform convulsions may occur, following which a change in the pattern of paralysis is frequently obvious.

The progressive changes in the clinical picture are no doubt associated with the spread of the lesions within the same hemisphere, to the contralateral hemisphere and to elsewhere in the central nervous system. Marked changes are generally associated with the development of

TABLE I

Duration of the Clinical Phases: The Age and Sex Incidence, the Nature of the Prodromal Illness and the Duration of Each of the Three Phases in the Reported Cases of Acute Hæmorrhagic Leuco-encephalitis

Author	Age (Years)	Sex	Prodromal Illness	Duration in Days				
				Prodromal Phase	Interval Phase	Neurological Phase	Neurological Phase	
							Precomatose	Comatose
Hurst (1941) Case 1 ..	33	F	—	—	—	2	½	1½
Case 2 ..	33	M	Respiratory infection	10	11	6	5	1
Henson & Russell (1942)	21	M	Respiratory infection	6	4	4	2	2
Shallard & Latham (1945)	7	F	Respiratory infection; measles in siblings	2	0	4	1	3
Wolf & Cowen (1946) ..	6	F	During course of pertussis immunisation	—	—	2	—	—
MacArdle *et alii* (1949) Case 1	22	M	—	—	—	4	—	4
Case 2	37	F	—	—	—	5	2	3
Fowler & French (1949)	37	F	Respiratory infection	5	0	3	1	2
Greenfield (1950)	51	M	Respiratory infection	?15	0	4	1	3
Moosy *et alii* (1954) ..	6½	M	—	—	—	—	—	—
Crawford (1954) Case 1 ..	28	M	Respiratory infection	4	6	6	2	4
Case 2 ..	2–10/12	M	Respiratory infection	2	0	7	1	6
Case 3 ..	31	M	Respiratory infection	3	0	7	3	4
Csermely & Haberland (1954) Case 3 ..	44	F	—	—	—	2½	½	2
Case 4 ..	50	F	—	—	—	11	3	8
Southcott & Fowler (1954)	33	M	Respiratory infection	3	6	10	4	6
Lander (1955)	36	M	Hæmorrhagic varicella; primary "viral" pneumonia	4	0	5	½	4½
Russell (1955) Case 1 ..	46	F	—	—	—	4	1	3
Case 2 ..	47	M	Respiratory infection	2	2	5	1	4
Aldridge (1956)	19	F	Respiratory infection	2	3	4	3	1
Rankin & Dance (1956) ..	50	M	Respiratory "infection" following tooth extraction	1	0	2	1	1
Symmers (1956) Case 1 ..	27	F	Oxophenarsine sensitivity (thrombotic microangiopathy)	—	—	—	—	—
Case 2 ..	13	F	Thrombocytopenia; splenectomy bronchiectasis; pneumonectomy infected wound	—	—	—	—	—

coma, but may occur only a few hours after the onset of the neurological phase.

Deviation of the head and eyes to one side usually occurs at a late state, and the development of unequal pupils and loss of the pupillary reaction to light generally just precedes death.

Examination of the fundus at an early stage seldom reveals any abnormality. Later, however, venous congestion, fundal hæmorrhages and papillœdema may be seen.

Urinary incontinence generally occurs early in the neurological phase and is not merely a late complication associated with the onset of coma. Urinary retention and fæcal incontinence may also occur early in the precomatose stage.

Other neurological signs which may be found include photophobia, trismus and neck stiffness. The latter may be of such a degree as to produce opisthotonos.

General Examination

General examination of the patient in this stage may reveal the persistence of the respiratory infection with the presence of sordes around the mouth and evidence of acute bronchitis or bronchopneumonia. Extrasystoles and soft systolic murmurs are not uncommon, and in the terminal phases a gallop rhythm may be heard. Considerable hepatomegaly early in the neurological phase has been noted on several occasions. Autopsy

TABLE II

Results of Investigations: Showing the Results of the Various Investigations Performed upon the Patients in the Reported Cases of Acute Hæmorrhagic Leuco-encephalitis

Case	Cerebrospinal Fluid								White Cells per Cubic Millimetre (% PMN)	Urine	Other Investigations and Comments
	Source of Fluid	Pressure in Milligrammes	Cells per Cubic Millimetres (% P.M.N.)	Protein (mg %)	Sugar (mg %)	Chloride (mg %)	Colloidal Gold Curve	Bacteriological Examination			
Hurst (1941) Case 2	—	—	—	—	—	—	—	—	22,900	—	—
Henson & Russell (1942)	LP	200	Clear	—	—	—	—	—	12,000 (82%)	—	—
	LP	260	Nil	40	—	—	—	—	—	—	—
	Vent.	—	6 (4 lympho-cytes)	35	—	—	—	—	—	Albumin ++++ No casts or red cells	—
Shallard & Latham (1945)	LP	Incr.	200 (90%)	30	65	763	—	Sterile	—	—	—
MacArdle *et alii* (1949) Case 2 ..	LP	—	6	55	78	—	Benzoin paretic type	—	—	Albumin +; a few red cells; no casts	Blood urea 30 milligram *per centum*
Fowler & French (1949)	LP	120	220 (95%)	125	100	—	—	—	—	—	—
	LP	—	1300 (95%)	—	—	710	—	—	8,500	—	—
Greenfield (1950) ..	LP	—	340 (84%)	60	62	760	—	Sterile	—	—	—
Crawford (1954) Case 1	Vent.	Incr.	220 (84%)	80	—	—	—	—	—	—	—
Case 2	LP		normal				—	—	30,000 (81%)	—	Performed during prodromal phase
Case 3	LP	—	Cloudy	270	—	—	—	—	—	—	—
	Vent.	—	150 (70%)	140	—	—	—	—	—	—	—
Csermely & Haberland (1954) Case 4	LP	—	" 30/3 "	60	—	—	Bis. Matrix. 0012343210 0	—	—	—	—
Southcott & Fowler (1954)	LP	210	430 (69%)	145	95	750	—	Sterile	24,800 (87%)	Albumin + 10 RBC HPF	Hæmoglobin 12·6 grammes *per centum*
Lander (1955) ..	LP	110	6 lympho-cytes	25	99	716	—	Sterile	15,500 (74%)	Albumin ++	—
Russell (1955) Case 1	LP	140	30	60	—	—	—	—	—	Albumin +	—
Case 2	LP	—	Nil	30	—	—	—	—	—	—	—
					—	—	—	—	22,500 (81%)	—	Hb 102%; ESR 80 in one hour
Aldridge (1956) ..	LP	300	2000 (90%)	620	80	720	—	Sterile	—	Albumin +++	Associated " nephrotic " nephritis. PM blood urea 124 milligrammes *per centum*
Rankin & Dance (1956)	LP	110	1400 (95%)	—	—	—	—	Sterile	—	—	—
	LP	100	Turbid	—	—	—	—	—	—	—	—

LP = Lumbar puncture
Vent. = Ventricular C.S.F.
Incr. = Increased

studies of the liver in these cases have shown only focal congestion. None of the case reports, however, have referred to the level of venous pressure.

INVESTIGATIONS

Examination of the cerebro-spinal fluid in the earliest stages may reveal no abnormality. Later, a rise in pressure generally accompanies an associated rise in the white cell count and protein content. The white cells found are nearly all neutrophilic polymorphonuclear cells. Colloidal gold curves have been performed in only two instances. In one case a paretic or first zone curve was obtained. In the other, a late midzone or meningitic-type curve was present. No alterations have been reported in either the sugar or chloride contents, and on those occasions in which it has been performed, bacteriological examination of the cerebro-spinal fluid has invariably given negative results.

The reported findings in the individual cases are shown in Table II.

The peripheral blood picture generally shows the features of a marked polymorphonuclear leucocytosis, and urinary examination not infrequently reveals the presence of protein, sometimes in considerable amounts.

Radiological views of the skull may show a shift of a calcified pineal gland, and displacement and distortion of the ventricular system may be seen if air studies are performed. Such findings, however, are seen only when one cerebral hemisphere is much more severely involved than the other. At autopsy the predominantly affected and grossly œdematous hemisphere may be seen herniated through the falx, above the corpus callosum.

In only one case (Henson and Russell, 1942) was a cerebral biopsy performed. In this instance, although the typical histological changes were evident in retrospect in the biopsy material, the condition was not recognized before death.

NATURAL HISTORY

An attempt has been made (Figure III) to summarize briefly in diagramatic form, the clinical picture and natural history of the disease, gained from a study of these 19 fatal cases.

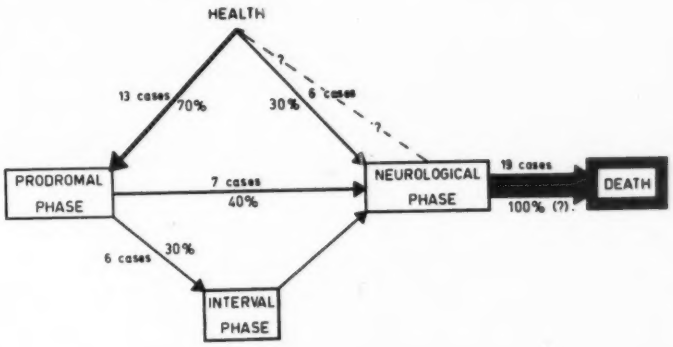

FIGURE III

The natural history of 19 of the 23 reported cases is illustrated in diagramatic form. The broken line represents the possible existence of non-fatal cases as yet undescribed under the title of acute hæmorrhagic leuco-encephalitis

The various phases are not always as clearly delineated as is suggested in the diagram. No reference is shown of the possible relationship of AHL to certain drugs and chemical agents, nor to other antecedent diseases such as varicella (Lander, 1955). Little provision has been made in the diagram for the possible existence of non-fatal cases, as no such cases have as yet been recorded under this title.

Nevertheless, it is suggested that this concept may be of value to the clinician when faced with a possible example of the condition.

AUTOPSY FINDINGS

At autopsy, the meninges may appear normal, although they are often congested, especially over the most severely affected hemisphere. In one instance (Aldridge, 1956) a thin layer of subarachnoid hæmorrhage was present around the midbrain, especially in the cerebro-pontine angles.

Macroscopically, the characteristic appearance is that of numerous small hæmorrhages scattered through the white matter of one or both cerebral hemispheres, affecting particularly the *centrum semiovale*. These hæmorrhages vary in size from "pin-point" up to areas a few millimetres in diameter, which in the more severely involved areas may be confluent.

Where the lesions are dense, œdema of the surrounding brain tissue is usually present, and this tissue may show a pinkish-grey discolouration. The most severely involved areas may be of almost gelatinous consistency, and even areas of softening and cystic change have been described. (Aldridge, 1956, Southcott and Fowler, 1954.)

As a rule, the middle third of the *centrum semiovale* is more severely involved than either the anterior or posterior thirds—the frontal and occipital poles are usually spared; and in general, the more centrally placed white structures (frequently including the *corpus callosum*) are more severely affected than the more peripheral white matter (Figure IV). Although the lesions often extend with increasing intensity into the white cores of the convolutions, the sparing of the subcortical U-fibres between adjacent convolutions has repeatedly emphasized.

In contradistinction to many other forms of encephalitis, the grey matter of the cerebral hemispheres remains almost invariably unaffected apart from vascular engorgement.

TABLE III

Autopsy Findings: Showing the Distribution of the Lesions of Acute Hæmorrhagic Leuco-encephalitis in the Central Nervous System. Other Autopsy Findings Together with the Results of Bacteriological and Viral Investigations Carried Out after Death are also Shown

Case	Cerebral Hemispheres	Cerebral Peduncles	Midbrain	Pons	Medulla	Cerebellum and Peduncles	Spinal Cord	Autopsy Studies: Bacteriological	Autopsy Studies: Viral	Other Autopsy Findings
Hurst (1941) Case 1 ..	L>R	×*	o	×	×*	×	—	—	—	—
Case 2 ..	L>R	o	×*	×*	o	o	—	—	—	—
Henson & Russell (1942) ..	Both	o	×*	o	o	o	o	—	—	"Inhalational" bronchopneumonia; focal congestion of liver; marked renal tubular degeneration
Shallard & Latham (1945)	L>R*	×	×	×	×	×	—	—	—	—
MacArdle *et alii* (1949) Case 1	L>R	×	×	×	o	×	—	—	—	—
Case 2	L>R	o	o	o	o	o	—	—	—	—
Wolf & Cowen (1946) ..	Right	o	o	o	o	o	—	—	—	—
Fowler & French (1949) ..	L>R	×	×	×	o	×	—	Negative	Negative	"Atypical" bronchopneumonia
Greenfield (1950)	L>R	o	o	×*	o	o	o	—	—	Hæmorrhagic bronchopneumonia
Moosy, Wolf & Cowen (1954)	—	o	Brainstem			o	—	—	—	Progressive diffuse cerebral sclerosis (dating from infancy)
Crawford (1954) Case 1 ..	L>R	o	×	×	o	o	—	—	—	Acute bronchitis
Case 2 ..	R>L	o	o	o	o	o	—	—	—	Pulmonary œdema and bronchopneumonia
Case 3 ..	Both	×	×	×	o	×	—	—	—	Bronchitis, bronchiolitis and bronchopneumonia
Csermely & Haberland (1954) Case 3	Both	o	o	×	o	×	×	—	—	Pulmonary œdema, subpleural and subpericaridal hæmorrhages
Case 4	Both	×	o	o	o	o	—	—	—	Left bronchopneumonia with effusion; chronic endocarditis and sclerosis of the aorta
Southcott & Fowler (1954)	L>R*	o	o	o	o	×*	—	—	Negative	Hæmorrhagic pulmonary œdema; [1]proliferation of plasma cell series in spleen and cervical lymph gland
Lander (1955)	Both	×	×	×	×	×	×	Negative	Negative	Hæmorrhagic varicella; bronchopneumonia (mononuclear); mild renal tubular degeneration; [1]proliferation of plasma cell series in spleen
Russell (1955) Case 1 ..	Both	o	o	o	o	o	o	—	—	—
Case 2 ..	R>L	o	o	o	o	o	—	—	—	Proliferation of plasma cell series in spleen
Aldridge (1956)	R>L	o	o	o	o	o	—	—	—	Large pale kidneys; "nephrotic" nephritis
Rankin & Dance (1956)	Both	×	o	o	o	×	o	—	—	Bronchopneumonia
Symmers (1956) Case 1 ..	Both	o	o	o	o	×	o	—	—	Widespread lesions of thrombotic thrombocytopenic purpura (also involving central nervous system)

× = Lesions present
* = Microscopic lesions only
o = No lesions present
— = Not examined or not mentioned
[1] = Findings in retrospect

The involvement of structures below the level of the cerebral hemispheres is variable. The pons and cerebellum have been involved in half the reported cases, although in several instances the involvement of these structures

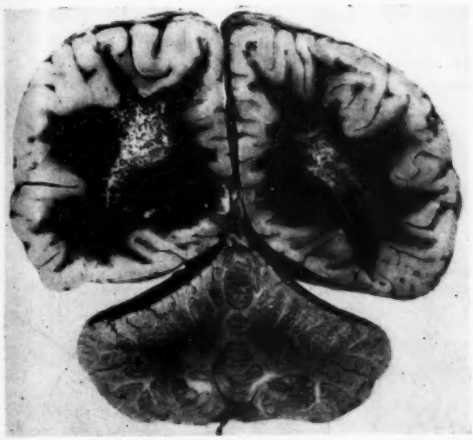

FIGURE IV

A coronal section of the brain at the level of the pineal gland from a case of acute hæmorrhagic leuco-encephalitis. Numerous punctate hæmorrhages are shown throughout the white matter of the cerebral hemispheres. Confluence of the hæmorrhages with pinkish-grey discolouration of the white matter is present towards the periphery. The subcortical U-fibres are spared. The cerebellar hemispheres are less heavily involved

was minimal. The spinal cord is rarely affected, although in a number of instances it was apparently not examined throughout its length. Involvement of the optic tracts was reported in the cases described by Fowler and French (1949) and by MacArdle *et alii* (1949—case 1). Lesions have not been recorded in other cranial nerves, nor in any peripheral nerve.

In regions other than the cerebral hemispheres, the predilection of the lesions for the white matter is not obvious. The distribution of the lesions in the cases reported is shown in Table III.

The macroscopic appearance of the brain is by no means specific, and the condition must be differentiated from such conditions as cerebral fat embolism and from other forms of "hæmorrhagic encephalitis". Brain "purpura" may mimic the appearance closely, but in this condition hæmorrhages may usually be found also in the grey matter.

Histology

The microscopic lesions are the diagnostic feature of this condition. They comprise hæmorrhages, perivascular exudates, changes in the walls of blood vessels and areas of demyelination and microglial proliferation.

The hæmorrhages occur in two forms: as "ball" hæmorrhages, often with a disrupted central capillary, and as "ring" hæmorrhages with a bloodless centre, which may or may not contain a fibrin-plugged capillary (Figure V). Where the lesions are of relatively long standing, disruption of the red cells and phagocytic activity may be seen.

The perivascular exudates usually occur around venules and are of two types. The perivascular space may be distended by either a serous exudate or by an accumulation of leucocytes, mainly neutrophilic polymorphonuclear cells (Figure VI). Lymphocytes and large

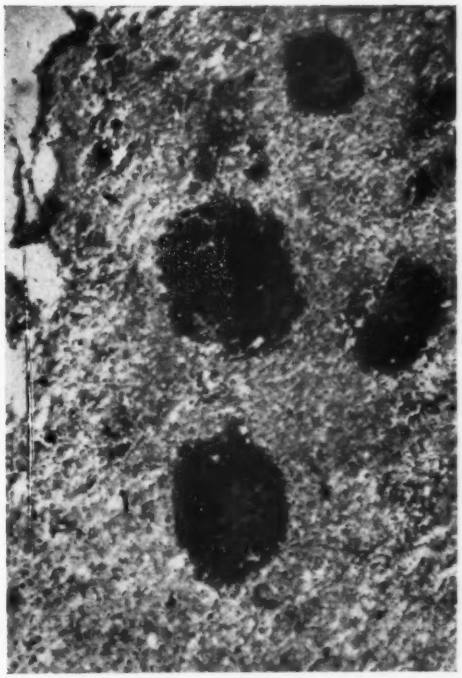

FIGURE V

A low-power photomicrograph showing adjacent "ball" and "ring" hæmorrhages in the white matter. (Hæmatoxylin and eosin stain. ×50)

mononuclear cells are usually also present in varying numbers, but eosinophils are not a feature of the condition. Exudates of mixed type are not uncommon.

The changes which occur in the walls of a varying number of blood vessels constitute

perhaps the most characteristic microscopic feature of the disease. The walls are impregnated or replaced by material which appears to be fibrin. This material often extends into the perivascular space and even

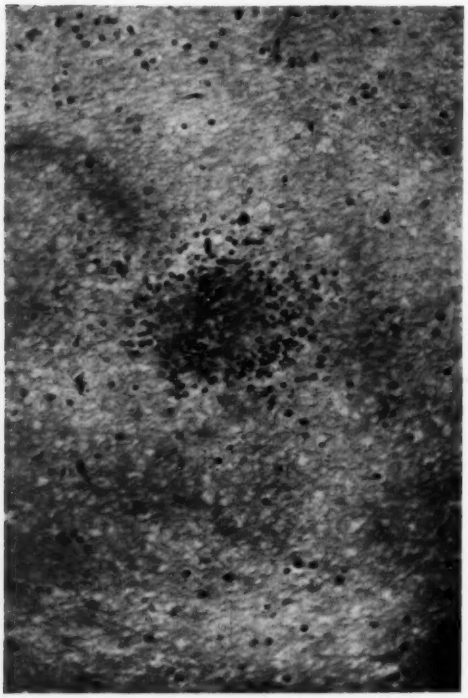

FIGURE VI

Photomicrograph of a perivascular lesion showing degeneration of the vessel wall together with cellular infiltration of the surrounding white matter. The majority of the cells are neutrophilic polymorphonuclear cells. (Hæmatoxylin and eosin stain. ×50)

into the surrounding brain tissue (Figure VII). Occasionally the material appears either to ensheath or to line a vessel, and the vascular lumen may be plugged by this substance. These changes generally affect the venules, but capillaries and even arterioles may also be involved. The areas of œdema, cellular infiltration and necrosis are frequently seen in relation to these involved vessels.

Small, focal and perivascular areas of demyelination are present more or less widespread throughout the involved areas (Figure VIII). Irregular patches are also found in and around areas of hæmorrhage. The exact relationship of these areas of demyelination to involved vessels has not as yet been adequately studied. Degeneration and disruption of neurofibrils in relation to many of the foci of demyelination has been demonstrated. Microglial proliferation and the presence of phagocytes containing fat can also be seen in many of these areas.

In the lungs, evidence of the original bronchitis, bronchiolitis or bronchopneumonia may be found. In other cases, consolidation or pulmonary œdema develops in the terminal stages, and death may be attributable to either event.

The presence in several cases of focal congestion in the liver, accounting for the hepatomegaly, has already been mentioned. Few references have been made to the

FIGURE VII

Photomicrograph of an involved venule showing fibrinoid degeneration of the vessel wall and strands of fibrin passing outwards into the surrounding white matter. (Phosphotungstic acid hæmatoxylin stain. ×140)

appearances in other organs, despite the not infrequent presence of proteinuria, often in moderate or even gross amounts. A noteworthy exception is the case described by Henson and Russell (1942), in which severe renal tubular

degeneration and necrosis were present. Lander (1955) also referred to the presence of striking tubular change, although in that instance the coexistence of hæmorrhagic chickenpox complicates the picture.

The presence of an excessive number of plasma cells and their precursors in the spleen is noted in one case of AHL and one of acute disseminated encephalo-myelitis described by Russell (1955). The spleen in the remaining

FIGURE VIII

A low-power photomicrograph showing small focal and perivascular areas of demyelination. (Weil's stain. ×50)

case of AHL described by Russell was not examined. Fowler (1957) has made similar observations in the spleen and in an enlarged cervical lymph node from the case he reported with Southcott in 1954. The writer has reviewed sections of the case he reported in 1955, and has found similar changes in the spleen.

DIAGNOSIS

To make a definitive clinical diagnosis in these cases is obviously difficult. Nevertheless, the condition should be considered when a case presents features similar to those described here.

When the lesions mainly involve one hemisphere, the commonest misdiagnosis is that of a cerebral abscess, either primary or secondary to the pulmonary condition. The diagnosis of cerebral thrombophlebitis may also be entertained in such cases.

On the other hand, when the lesions are spread diffusely throughout both hemispheres and elsewhere in the brain stem, the diagnosis of "encephalitis", without qualification, or of "purulent meningitis", may be made. Sometimes parainfectious encephalitis has been diagnosed, e.g. following influenza (Greenfield, 1950) and chickenpox (Lander, 1955).

In a condition such as this, in which exploratory craniotomy is often undertaken, it is suggested that cerebral biopsy should always be performed. This would offer a method whereby a definitive diagnosis could be established before death. It is possible that the existence of non-fatal cases would be demonstrated. Detailed clinical study might then result in a considerable increase in our knowledge of both this and related diseases. A lead in this direction has recently been given by Kristiansen and his colleagues (1956). Furthermore, the establishment of an early diagnosis would allow some attempt at therapy.

AETIOLOGY

The ætiological possibilities which have been considered include: (i) invasion of the brain tissue by bacteria or virus, (ii) direct action of circulating toxic substances, and (iii) acquired sensitivity of brain tissue to a circulating antigen.

Infection

It would appear unlikely that the condition is a result of either direct bacterial or viral invasion of the central nervous system.

In those cases in which bacteriological examination of the cerebro-spinal fluid has been performed, it has been sterile. Bacteriological and viral studies of autopsy material have also given negative results in the few cases in which they have been carried out.

It is still possible, however, that a virus may yet be incriminated. The coexistence of AHL and hæmorrhagic chickenpox in one case (Lander, 1955), of atypical ("Viral") pneumonia in another (Fowler and French, 1949) and of the occurrence of measles in the siblings of the female child described by Shallard and Latham (1945), might be cited as superficial evidence in support of this view.

However, the pathological findings in AHL are quite different from those resulting from known virus infections.

Action of Toxic Substances

This possibility is supported by the knowledge that a number of poisons such as carbon monoxide, potassium cyanide and sodium azide (Hurst, 1942) can produce demyelination in certain experimental animals. These agents also produce necrosis. Substances such as ergot, saponin, bee venom and tetanus toxin can also experimentally cause demyelination (Hurst, 1944). However, caution is necessary in applying the results of animal experimentation to the concepts of human disease, and in this connection the marked rarity of demyelinative lesions in human tetanus is recalled.

It has been argued (Hurst, 1944; Morrison, 1946; Hurst, 1952) that the lesions produced by such noxious agents are due to anoxia mediated by vascular spasm, and it is well recognized that the effects of hypoxia are most marked in the white matter.

Furthermore, anoxic effects could be expected to be most marked at the level of venules. Thus, at least superficially, the distribution of the lesions in AHL might well be explained by the action of a toxic substance.

Clinically, the occurrence in a number of cases of such manifestations as proteinuria, renal tubular damage, hepatomegaly and cardiac disturbances might be considered as evidence in support of a toxic ætiology. Thus this possibility, although unlikely, cannot be positively excluded.

Acquired Sensitivity to a Circulating Antigen

A third possibility—that of acquired sensitivity to a circulating antigen—is the popular view at present. It has many points in its favour, and much evidence is accumulating in support of it.

On the clinical side, the frequent onset of the neurological disturbances some days after a respiratory infection is consistent with the time interval observed with other hypersensitivity states.

Wolf and Cowen (1946) described the development of AHL in a female child during a course of prophylactic pertussis vaccination. Symmers (1956) described two cases of great interest. One was that of a young woman, who developed primary syphilis, which was treated with penicillin and oxophenarsine hydrochloride. Seven years later she developed Vincent's gingivitis and was again given oxophenarsine locally. Immediately, she developed a severe generalized hypersensitivity reaction, which subsided on administration of adrenaline.

A few days later she developed an acute febrile illness, with hæmolytic anæmia, thrombocytopenic purpura and fluctating neurological abnormalities. A diagnosis of thrombotic thrombocytopenic purpura was made.

At autopsy, this diagnosis was substantiated, but a surprising finding was the presence of the typical lesions of AHL. Thus is seen in the one case the coexistence of hypersensitivity, the collagen disease of thrombotic thrombocytopenic purpura, and the lesions of AHL. In his second case there was once again a very great possibility that the terminal leuco-encephalitis was the result of sensitization to drugs or microbial allergens.

Histologically, similar lesions have been described in arsphenamine encephalitis (Russell, 1937)—a supposed sensitization phenomenon, and in a patient with hypersensitivity following the administration of streptomycin and para-amino-salicylic acid (Cavanagh, 1953).

Crawford (1954) and others have pointed out the close resemblance of the vascular lesion to that occurring in *polyarteritis nodosa*, and the similarity to those produced experimentally in animals by the repeated injection of foreign protein (Klinge, 1930; Clark and Kaplan, 1937; Rich, 1942). In 1955, Russell described three cases illustrating the thesis that AHL and acute disseminated encephalo-myelitis represent variants of the one nosological entity. In the case of acute disseminated encephalo-myelitis described, an arteriolar lesion of *polyarteritis nodosa* type was present. However, it must be noted that the vascular lesion in the above disorders occurs rather on the arterial than on the venous side of the circulation.

Many workers (Rivers *et alii*, 1933; Rivers and Schwentker, 1935; Morgan, 1946; Kabat *et alii*, 1946, 1947, 1948, 1949; Wolf *et alii*, 1947, and others) have shown that the demyelination can be imitated experimentally in animals by single or multiple injections of emulsions of brain tissue, with or without certain adjuvants (after Freund and MacDermott, 1942). Vascular inflammatory lesions and even hæmorrhage and softening are also produced by these methods (Morgan, 1946, 1947; Wolf *et alii*, 1947). The resulting lesions are confined mainly to the white matter, and the illustrations of the macroscopic lesions are reminiscent of AHL (Hurst, 1952). The general consensus of opinion, however, is that the histology of this experimental condition resembles more closely that of acute disseminated encephalo-myelitis (Lumsden, 1956; Colover, 1957).

If the condition of AHL is a manifestation of a hypersensitivity state, the question of the nature of the allergen is raised.

The concept of autosensitization to the products of bacterial or viral invasion, and to the administration of certain drugs and chemical compounds, has been frequently invoked in certain other demyelinating conditions. This concept would appear to be the most likely one in the present state of our knowledge, although the mechanism of the changes remains virtually unknown. Lumsden (1956) has summarized existing knowledge on the subject.

RELATIONSHIP TO OTHER DISEASES OF THE CENTRAL NERVOUS SYSTEM

A. *To Other Types of "Hæmorrhagic Encephalitis"*

In his original paper in 1941, Hurst maintained on almost purely histological grounds that AHL was a distinct entity. He differentiated the condition from such disorders as brain purpura (Schmidt, 1905) and Baker's hæmorrhagic encephalitis (Baker, 1935).

In 1949, Adams *et alii* described a very similar disorder under the title of acute necrotizing hæmorrhagic encephalopathy. In the four cases described the patients all had a prodomal respiratory infection and all died within 30 to 60 hours of the onset of neurological features. A marked polymorphonuclear leucocytosis was present in the cerebro-spinal fluid in the two cases in which it was studied. Although the lesions reported were very similar to those described by Hurst, the cerebral white matter was spared in two cases. Viral studies in one case gave negative results. In one of their cases, a white cell count in the cerebro-spinal fluid of 5,000 per cubic millimetre (100% polymorphs) was associated with the finding of pyogenic cocci in the brain tissues.

These authors reviewed the literature on the various types of hæmorrhagic encephalitis, affecting chiefly the white matter, which had been described. They noted the similarity of their cases to those described by Hurst (1941) and by Henson and Russell (1942). They also noted that Margulis *et alii* (1944) believed that they had isolated a filterable virus from the blood, cerebro-spinal fluid and brain tissue in similar cases. Adams *et alii* (1949), however, favoured an allergic hypothesis.

In 1952, Adams and Kubick reviewed the pathological aspects of several demyelinating diseases. They considered that AHL and a number of cases of encephalitis described under a variety of titles by Gayet* (1875), Leyden* (1881), Strumpell* (1891), Bückler* (1892), Brie* (1897, Oppenheim (1897), da Fano (1924), Baker (1935), and Levy (1937) were but variations on the theme of acute necrotizing hæmorrhagic encephalopathy.

In 1954, Southcott and Fowler maintained that case IV of Adams *et alii* (1949) was an example of AHL. They rejected the other three cases (Fowler, 1957) on the grounds of the lack of cerebral hemisphere involvement in two cases, and the presence of pyogenic cocci in the brain tissues of the third.

Also in 1954, Crawford published his review of the pathological features in seven reported cases and added three of his own. He did not refer to the cases described by Wolf and Cowen (1946) and by Fowler and French (1949). He concluded that descriptions of the cases published up to that time strengthened Hurst's contention that AHL was indeed a specific disease entity distinct from other forms of encephalitis.

In 1956, a Norwegian group led by Kristiansen described under the title of acute hæmorrhagic encephalitis (after Strumpell, 1891) a collection of five cases with essentially the same clinical and pathological features as those presented in this paper. In four of these cases, the diagnosis was made by cerebral biopsy. In two of these cases the patient survived. In one of the survivors a considerable degree of recovery occurred, but the other exhibited little improvement following the acute stage of his illness.

These workers suggested that the conditions described by Hurst, Adams *et alii*, Baker and a number of other investigators (including the inclusion encephalitis of Malamud *et alii*, 1950) were but variations on the basic pathological picture of acute hæmorrhagic encephalitis.

Thus the position is confused. It would appear that a number of very similar cases have been reported under a variety of titles, and that in the past more attention has been paid to differences rather than to points of similarity between these cases. Nor has the problem been simplified by the frequency of reports containing inadequate clinical or pathological details. At the present time, Kane (1957) is probably correct when he states that "the diagnosis, which has many synonyms . . . is commonly called 'acute hæmorrhagic leuco-encephalitis' in Australia and England, and 'acute necrotizing hæmorrhagic encephalopathy' in . . . the United States".

B. *To Other Demyelinating Conditions*

Hurst also suggested that AHL bore some relationship to the demyelinating diseases. Wolf and Cowen (1946) were probably the first

* Cited by Adams and Kubick (1952).

to suggest that AHL was a variety of disseminated encephalo-myelitis. Csermely and Haberland (1954) and Russell (1955) described supportive evidence, and it is possible that the case described by Lander (1955) of the coincidence of AHL and chickenpox adds some strength to the concept of the nosological unity of AHL and acute disseminated encephalomyelitis (Lancet, 1956). A further link with the demyelinating conditions was suggested by Moosy *et alii* (1956) on the basis of the coexistence in one patient of progressive diffuse cerebral sclerosis dating from infancy and the lesions of AHL.

Is it to be concluded then that these various " hæmorrhagic encephalitides ", AHL and such demyelinating conditions as acute disseminated encephalo-myelitis are but variants of the one neuropathological entity ?

It is submitted that no didactic conclusions are possible at present. The difficulties of classification and interpretation are many. These are accentuated by the fact that brain tissue is able to respond to injury in only a limited number of ways ; that similar histological lesions are not necessarily of similar pathogenesis ; and that the same disease may give rise to different histological appearances at different stages in its evolution.

These problems are probably not insoluble, but it would appear that little help can be obtained from past observations. They can possible best be tackled in the future by a careful correlation of clinical and pathological data, the latter being obtained not only at autopsy, but during life by increased use of cerebral biopsy methods. A start in this direction has been given by Kristiansen (1956) and his colleagues.

Treatment

If AHL is due to hypersensitivity as has been suggested, it is submitted that a trial of cortisone (or related compounds) should be considered in the diagnosed case.

Such treatment would have to be given early, however, for experimentally induced " allergic " encephalo-myelitis can only be prevented or ameliorated when treatment is instituted soon after the inoculation of the allergen (Good *et alii*, 1949 ; Moyer *et alii*, 1950).

Experience in the apparently allied disease of human disseminated encephalo-myelitis substantially bears out these experimental findings. Approximately two-thirds of such cases treated with ACTH (or cortisone) have definitely been benefited, and in general the results are better if such treatment is initiated early in the course of the disease. (Garrison, 1952 ; Miller, 1953*b* ; Miller and Gibbons, 1953, 1954 ; and Selling and Meilman, 1955.)

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ADDENDUM

Since this paper was submitted for publication, two further relevant contributions have appeared in the literature.

Stevens (1957) reports a case of acute hæmorrhagic leuco-encephalitis which developed suddenly, without preceding ill-health, in a woman of 44 years. Urinary incontinence, cyanosis and spastic quadriplegia developed rapidly, and death occurred on the third day. At autopsy, the typical lesions were found in both cerebral hemispheres and in the brain stem. Viral studies on the spinal cord were unrewarding.

Two cases of " hæmorrhagic encephalitis ", in which similar lesions were found predominantly in the white matter, are also described. Polymorphonuclear infiltration was absent in these cases. The terminal illness followed shortly after a respiratory infection in one case, and after the alimentary administration of *liquor arsenicalis* in the other. The similarity between the three cases is emphasized.

McLetchie and Stevenson (1957) describe two cases under the title of " Allergic Encephalitis ". They emphasize the value of aspiration biopsy in the diagnosis, which was confirmed at autopsy in the first case. In the other, a man of 21 years, the illness ran a subacute course lasting two months. Prompt and complete recovery followed the administration of cortisone in this instance.

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THE SIGNIFICANCE OF PULMONARY VASCULAR CHANGES IN EMPHYSEMA[1]

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SUMMARY

Observations on the morphology of vascular changes in emphysema and related conditions are presented, with particular emphasis on those in the pulmonary and bronchial arteries.

These observations indicate that the sclerosis of the small arteries in emphysema results from organization of thrombi, this thrombosis being precipitated by the cause of the inflammation of the adjoining air passages producing some degree of damage to the arterial wall. In early generalized emphysema pulmonary arterial sclerosis is found predominantly in the small arteries adjoining terminal and respiratory bronchioles; in these early lesions inflammatory damage is largely confined to these bronchioles.

It is emphasized that sclerosis of the smaller pulmonary arteries is not directly proportional to the degree of inflammatory damage to bronchioles. The relation between the two, particularly in early or mild cases, varies within wide limits.

It is pointed out that the significance of pulmonary arterial sclerosis as one factor in the genesis of pulmonary hypertension has not yet been accurately assessed, since morphological studies of this subject have been limited and the widespread complete arterial obliteration that occurs in chronic inflammatory disease of the lungs has not been adequately recognized.

Observations on the bronchial arteries in emphysema and on the development of anastomoses between these vessels and pulmonary arteries indicate that these last develop from the minute vessels in organizing tissue in inflammatory areas. Where a " demand " for nutriment continues, the vessels enlarge. Such vessels are bronchial in origin; and where the wall of a pulmonary vessel has been involved, an anastomosis arises.

The vascular changes observed in emphysema are not of great ætiological importance but are the incidental concomitant of earlier general connective tissue changes in the region. As happens in all parts of the body, the vessels are necessarily involved in any connective tissue disturbance. At the same time sclerosis and particularly obliteration will limit the nutrition of tissues depending on them.

The vascular phenomena therefore are important in that, by their presence, they support the hypothesis propounded, as the result of observations of the bronchioles and related structures, that emphysema is intimately related to and develops as the result of previous damage to and chronic inflammatory changes in the bronchioles and proximate pulmonary tissues.

According to the variety of ground, the same mischief was produced by different means.
Edward Gibbon: *The Decline and Fall of the Roman Empire*, Chapter LXXI.

THE structural changes in the pulmonary blood vessels in emphysema, and in related conditions such as bronchiectasis, have been examined previously many times and from many aspects, so that it is astonishing that one particular feature has been largely neglected. This is the relation of changes in pulmonary vessels to inflammatory damage in the adjoining tissues. It is this relation that is stressed here, especially the changes in the small arteries adjacent to those bronchioles showing evidence of past acute bronchiolitis.

MATERIALS AND METHODS

Radiographic studies on the bronchial arteries were carried out on the lungs of 22 patients, including 12 with moderate or advanced emphysema. Two of these also showed gross bronchiectasis, which was also present in three of the patients without significant emphysema. The lungs in two patients with severe mitral stenosis and in five that were macroscopically normal were also examined. The thoracic viscera were removed *en bloc* by the method of Cudkowicz and Armstrong (1951), and the

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lower part of the thoracic aorta was cannulated. The heart was removed and the ascending aorta ligated along with the branches of the arch. Compressed air was then blown through the cannula to make the cut ends of intercostal arteries easily recognizable; all vessels leaking air were ligated.

The radioopaque material used was 60% "Lipiodol" in water, emulsified by the addition of a detergent—8% Tween 80. This fluid was injected with a large syringe into the aorta, the filling of the bronchial arteries being followed by examination under a fluorescent X-ray screen. The emulsified lipiodol flowed readily into the finest vessels, but was sufficiently viscous to permit temporary interruption of the injection and exposure of X-ray films. It was usually necessary to keep the lungs expanded; this was done by cannulating the trachea and applying a slight constant flow of compressed air.

Histological examination was made of parts of these lungs and also of other lungs in which the bronchial arteries had been isolated and injected with Indian ink. Some of this material was embedded in celloidin, but with most the paraffin method was used.

The relation of vascular changes to damaged bronchioles and bronchi was studied in specially prepared material which was serially sectioned. This material and the methods used have been described previously (McLean, 1956*b*). In addition approximately 50 blocks were cut from 34 bronchiectatic lobes removed at operation. Several sections were cut from each block, and two blocks were completely serially sectioned. The histological features are described first.

OBSERVATIONS

Pulmonary Vessels

1. *The Large Arteries.*—Atheroma was commonly seen in the main pulmonary arteries and in their lobar and segmental branches, and in all subjects over 40 years of age at least some flat yellow patches of atheroma were found. In 15 cases of macroscopically normal lungs and in 15 cases of emphysema the individual ventricles were separated and weighed. In those with more severe atheroma, in which the plaques were obvious even on cursory examination, it was clear that the extent of these lesions was related more to the degree of atheroma in the systemic arteries than either to the severity of the emphysema or to the weight of the right ventricle.

2. *The Small Intrasegmental Arteries.*—In normal lungs these arteries were thin walled; a single layer of endothelial cells formed the intima. The musculo-elastic media showed laminæ of elastic tissue in the larger vessels, but in the smaller arteries only two layers, the internal and external elastic laminæ, remained (Figure I). Further peripherally the vessel lumen tapered evenly, and approximately at the level of the third order respiratory bronchiole of the alveolar ducts, the elastic laminæ were no longer recognizable and few muscle fibres could be demonstrated. Subsequent branches differed essentially from capillaries only in the size of their lumens.

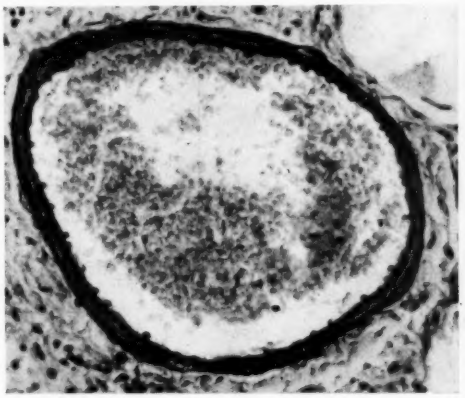

FIGURE I

Photomicrograph showing a normal small artery adjacent to a medium-size bronchiole in a child's lung. Most of the concentrically arranged elastic tissue is condensed into two layers—the internal and external elastic laminæ. (Elastin stain, ×14)

Serial sections of tissue from a number of macroscopically normal lungs were examined. Many of these lungs showed generalized emphysema histologically, and in these there was evidence of old damage to the bronchioles, which was greatest in the terminal and the first and second order respiratory bronchioles (McLean, 1956*b*); the degree of emphysematous change approximately paralleled the degree of bronchiolar damage (McLean, 1957*b*, *c*).

On the histological appearances it was possible to grade these lungs ranging from examples with no histological emphysema to those with obvious lesions which could, in fact, be recognized on careful macroscopic examination.

In several the small arteries generally appeared normal, only occasional vessels showing slight intimal thickening. However, in others intimal thickening was commonly present, and in a few lungs this was readily demonstrable in

most of the smallest arteries; at the same time intimal thickening was rarely severe enough to produce a significant reduction in the size of the lumen. In these examples the thickened intima contained numerous elastic fibres, sometimes obscuring the demarcation of the internal elastic lamina of the media. Nevertheless, the media was usually recognizable but often showed patchy destruction of both its elastic and muscular elements, particularly where the vessel wall was related topographically to tissue showing evidence of old inflammation (Figure II).

FIGURE II

Photomicrograph showing a small artery adjacent to a damaged respiratory bronchiole in a lung in which emphysema was visible only histologically. The intima is thickened and contains numerous densely matted elastic fibrils. The media is not present at one site (above). Ciliated epithelium (unusual in such passages) rests on a slightly irregular subepithelial elastic net. (Elastin stain, × 100)

That these sclerotic changes were largely restricted to the small arterial branches accompanying terminal bronchioles and respiratory bronchioles seemed significant, especially since arterioles distal to the third order of respiratory bronchioles as well as larger intrasegmental arteries were normal. The intimal thickening was usually almost symmetrical, but occasionally was greater on one side of the lumen than the other. The degree of change was generally greater in the vessels of a more damaged secondary lobule than in those of less affected adjacent lobules.

Much of this tissue was fixed *in situ* by intravenous perfusion of the cadaver (McLean, 1956*b*), and the pulmonary vessels, compared with the size found, for instance, after endobronchial injection of fixative, were considerably dilated. In tissue fixed by intravenous perfusion, the approximate average measurements of a normal artery corresponding to a second order respiratory bronchiole were: internal diameter 200–300μ and wall thickness 5–8μ. In these circumstances the elastic fibres were straight and not, as is customary, wrinkled: this suggests that this size approximates to that occurring *in vivo*. On the other hand, similar vessels showing slight sclerosis, even in the same lungs, were considerably smaller. The artery in Figure II was typical of this group—its internal diameter was 110μ and the wall thickness 25–50μ. Although these observations give only an indication of the situation during life it should be clear that the functional effects of intimal sclerosis are not due solely to encroachment on and narrowing of the lumen, but are also attributable to decreased distensibility.

In lungs showing macroscopic focal emphysema extensive and gross sclerosis of these small arteries was not seen, but slight intimal thickening, inconspicuous without elastic stains, was usually recognizable. On the other hand, in severely emphysematous regions of more damaged lungs small arteries were always obviously sclerosed, but the change was considerably greater in some cases than in others. Larger intrasegmental arteries, which were affected slightly or not at all in less emphysematous lung, sometimes were also involved extensively. The greatest sclerosis of intrasegmental pulmonary arteries was seen in vessels accompanying ectatic bronchi or bronchioles, in which the adjacent tissue showed much evidence of old inflammation, and in those within "scar" tissue, that is to say, within condensed and organized lung.

Sclerosis of these more damaged arteries was also largely due to intimal thickening, usually consisting of fibrillary eosinophilic material containing occasional ovoid nuclei. Dense elastic fibre formation was common, especially in examples in which the thickening was slight; collagen fibres were demonstrable only in vessels with considerable old damage to the media. On rare occasions smooth muscle could be identified in the thickened intima as isolated cells (or even as the predominant tissue) arranged approximately longitudinally. Muscle cells were somewhat more commonly seen in sclerosed pulmonary arteries in the bronchiectatic lobes of young people than in emphysematous lungs.

FIGURE IIIA

Photomicrograph showing part of the thickened wall of a tortuous pulmonary artery adjacent to a heavily damaged ectatic bronchiole in an emphysematous area of a bronchiectatic lobe. With hæmatoxylin and eosin staining (A) the lumen (right) is bordered by a smooth rounded and endothelium-lined surface.

FIGURE IIIB

Same section as IIIA. With elastin stain (B) the original internal elastic lamina is apparent, most of the convexity being due to thickened intima. An obliterated arterial branch is seen, which was traced peripherally for several millimetres until its remnants could no longer be discerned. (×90)

FIGURE IV

Photomicrograph showing a small vein in a residual strand of tissue in a region of advanced emphysema. The lumen is almost obliterated by intimal thickening. A small tributary (lower right) has been completely occluded. (Elastin stain, ×120)

FIGURE V

Photomicrograph of a thick (100μ) section of part of the wall of a bronchus in a normal lung in which the bronchial arteries were injected with indian ink. The rich vascular supply of the bronchial wall is shown, particularly in the subepithelial region. (×30)

In regions showing extensive old damage, completely obliterated pulmonary arteries were frequently recognized by the residual elastic tissue of their walls. However, only vessels that must originally have been of large size were easily identified. Some were seen in scars which obviously included obliterated bronchioles, and others were recognized in the walls of relatively large pulmonary arteries accompanying ectatic large bronchioles (Figure III). In either case the remnants of the arteries could be traced for a short distance into the abundant periarterial scar tissue (in which traces of obliterated bronchioles were often recognizable), but it was not found possible to recognize the remnants of their finer divisions.

Similar complete obliteration of arteries of the size of those normally found adjacent to terminal and respiratory bronchioles was never recognized with certainty, although, as in the cases just mentioned, it was clear that some had been obliterated. Thus it is evident that these vessels can be obliterated and leave only inconspicuous remnants.

3. *Capillaries.*—It is manifest that in some of the severe degrees of emphysema, where there is diminution in the amount of pulmonary tissue (McLean, 1957*d*), there is considerable reduction in the pulmonary capillary bed, but observations were not made which indicated the precise manner in which the disappearance occurs.

4. *Veins.*—In the early stages of emphysema, even in lungs with readily demonstrable pulmonary arterial sclerosis, no significant sclerotic changes were seen in the pulmonary veins. With further progress of the disease, however, veins were often involved, the lesions being similar to those found in the arteries (Figure IV).

Bronchial Vessels

1. *Bronchial Arteries.*—These relatively small vessels vary in number and in their origin. Commonly one left artery arises from the aorta and two right bronchial arteries arise from intercostal arteries.

The branches of these arteries course peripherally in the outer wall of bronchi and, except for the alveolar walls, supply all the tissue of the lung, including bronchi (Figure V), bronchioles, and walls of pulmonary arteries and veins (Cudkowicz and Armstrong, 1951).

In emphysema constant histological changes in the bronchial arteries were not found; in many lungs showing early or moderately advanced change they appeared normal. In patients with severe systemic hypertension slight sclerosis of the bronchial arteries was sometimes recognized in the larger arteries, but this change was independent of the presence of emphysema.

Easily recognizable sclerosis was seen only in arteries in the walls of bronchi showing plentiful evidence of old inflammation. Such arteries were seen in severely emphysematous lobes, in the walls of ectatic bronchi; sclerotic vessels were common in bronchiectasis, in which few bronchial arteries were normal. Many of these vessels often were much increased in diameter, but most showed some intimal thickening, which was occasionally severe. The intima frequently contained elastic fibres and, particularly in bronchiectatic lobes, muscle fibres arranged longitudinally (Figure VI). Occasionally such musculo-elastic change in the intima was found in many bronchial arteries within a bronchiectatic lobe; the media often appeared normal.

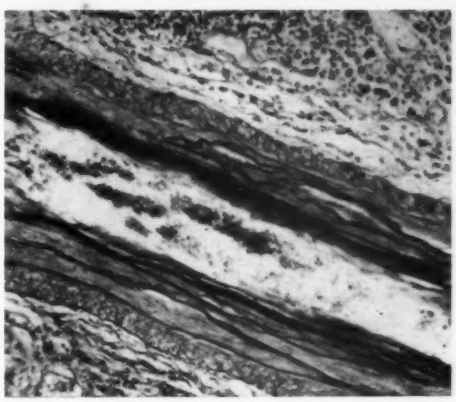

FIGURE VI

Photomicrograph showing a sclerosed bronchial artery in the wall of an ectatic bronchus. The media is demarcated from the relatively thicker intima, which contains numerous longitudinal elastic fibres. With appropriate staining, smooth muscle fibres were demonstrated in the intima in adjacent sections. (Elastin stain, ×60)

2. *Capillaries and Veins.*—The bronchial arteries normally break up into a net of capillaries surrounding the bronchus and supplying the structures of the wall. Small branches lead to the walls of pulmonary vessels as *vasa vasorum*. The peribronchial net continues peripherally, and, in the region of the respiratory bronchioles, these capillaries become continuous with others derived from pulmonary arterioles. In emphysema there

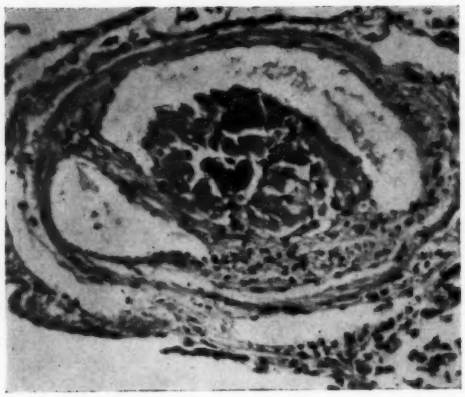

FIGURE VII

Photomicrograph showing a small mass of recent thrombus attached to a strand of older thrombus crossing the lumen of a small artery in the wall of respiratory bronchiole. These thrombi were probably of thrombo-embolic origin. The recent thrombus is showing early organization with phagocytosis of the amorphous eosinophilic material forming the thrombus. (Hæmatoxylin and eosin, ×100)

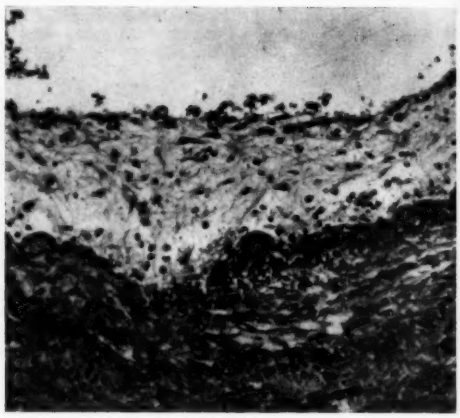

FIGURE VIII

Photomicrograph showing the wall of a pulmonary artery of three millimetres diameter in a case of staphylococcal pneumonia. The internal and external elastic laminæ can just be discerned as dark bands. Overlying the internal lamina is a layer of loose reticular connective tissue representing organizing thrombus. Most arteries in this lung of similar or smaller size showed corresponding change, many being completely occluded; numerous small infarcts were recognizable. (Hæmatoxylin and eosin, ×90)

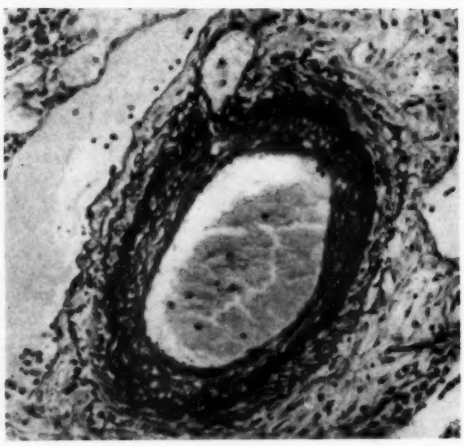

FIGURE IX

Photomicrograph showing a small pulmonary arterial branch adjacent to a recently damaged and ectatic bronchiole. A dilated lymph vessel is shown (left). A small bronchiolar vessel (upper left) enters the thickened intima of the pulmonary artery and communicates with the lumen in adjacent sections. The media of the artery is extensively damaged on the side nearer the bronchiole (left). (Elastin stain, ×90)

FIGURE X

Photomicrograph showing part of a bifurcation of an artery. A strand of organized thrombus extends across the lumen and into one branch (to the upper left). This new tissue contains small capillaries; under higher magnification tiny granules were seen in the organized thrombus, which gave a positive reaction for ferric iron with the Prussian blue test. This tissue is clearly continuous with "thickened intima" in the wall (above centre). (Hæmatoxylin and eosin, ×60)

was little change in the capillary net except that it was less regular than normal around damaged bronchioles. Near the hilum the venous drainage of the net is into the bronchial veins; these veins were not examined in this study. Farther peripherally in the lung the bronchial capillaries are drained by vessels which empty into pulmonary veins. In serial sections, these venules were often observed to arise in the region of lymphatic aggregations at the bifurcations of bronchioles and to travel several millimetres before entering pulmonary veins. Their number and size appeared to be similar in normal and moderately emphysematous lungs.

The Evolution of Arterial Sclerosis and Interarterial Anastomoses

During this study several observations, which were consistent with views expressed by others, were made on pulmonary arterial sclerosis. In sections from several lungs a recent thrombus was found partially or completely occluding small pulmonary arterial branches. This was observed both in inflammatory lesions, particularly in staphylococcal pneumonias, and in non-inflammatory pulmonary thrombosis and embolism (Figure VII). In cases in which the thrombus did not completely fill the lumen, its surface had often been covered by endothelium before the homogeneous eosinophil material representing the clot was changed. Phagocytes, probably including many of endothelial origin, were then seen in the thrombus, and at this stage the amorphous material was no longer apparent. Gradations were observed between these phagocytic cells and cells of an ovoid shape surrounded by intercellular substance. At this later stage the thrombus consisted of a loose network of cells separated by faintly basophil and loosely fibrillary intercellular substance (Figure VIII). In examples that were obviously inflammatory in origin, the media often showed patchy destruction of muscle and elastic tissue. Subsequently, both inflammatory and non-inflammatory lesions showed numerous thin-walled capillaries penetrating the media of the vessel and entering this new tissue (Figure IX); it was clear that many completely occluded arteries had been recanalized by proliferating capillaries derived from the *vasa vasorum*—branches of bronchial arteries—thus forming small interarterial anastomoses. The residual tissue within the lumen therefore usually lined the wall, but in several examples the lumen of small pulmonary arteries was crossed by an interlacing perforated mesh of tissue indistinguishable in appearance from that of thickened intima (Figure X).

In these cases collagen and elastic fibrils were sometimes demonstrable in the layer of organized thrombus. At this stage many lesions were identical with typical arteriosclerotic change, and the origin from thrombus was recognizable only in those showing features such as strands of tissue crossing the lumen as in the example just described.

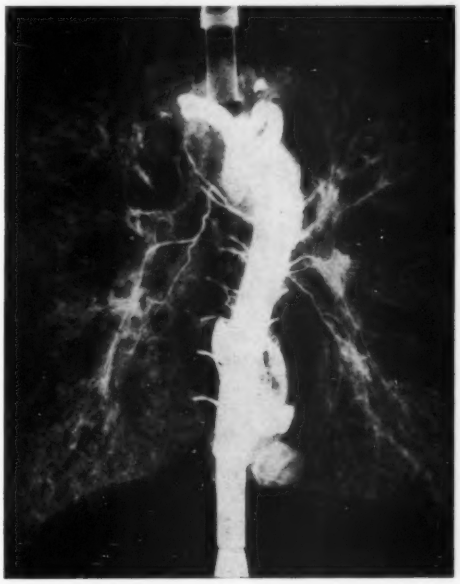

FIGURE XI

X-ray photograph of macroscopically normal lungs in which the aorta was injected with radioopaque material. The bronchial arteries are of normal size and distribution. A single right bronchial artery is seen arising from an intercostal artery. The arterial branches become indiscernible within six to eight centimetres of the hilum.

Anastomoses Between The Two Circulations

As has been mentioned, there are extensive capillary communications between the bronchial and pulmonary circulations, particularly in the region of terminal bronchioles. More direct anastomotic channels connecting the two circulations have been described in macroscopically normal lungs, both between bronchial arteries and pulmonary veins (von Hayek, 1940*a*) and between bronchial and pulmonary arteries (Küttner, 1878).

The interarterial anastomoses have been found to increase considerably in many conditions, and it is these communications which have been specially studied.

The bronchial arteries in macroscopically normal lungs showed gradual tapering of parabronchial branches (Figure XI): in such lungs

FIGURE XII

X-ray photograph showing the upper half of a right lung in which the bronchial arteries were injected, as in Figure XI. At the apex there was a dense scar, superficial to which were several small air cysts. A tortuous subpleural bronchial artery leads to the region of the scar, where two pulmonary arteries show retrograde filling through interarterial anastomoses.

FIGURE XIII

X-ray photograph illustrating the apical air cysts shown in Figure XII, after further injection of radioopaque material. A tortuous bronchial artery extends (from the right) over the surface of an air cyst, eventually communicating with a pulmonary vein (below cyst on left).

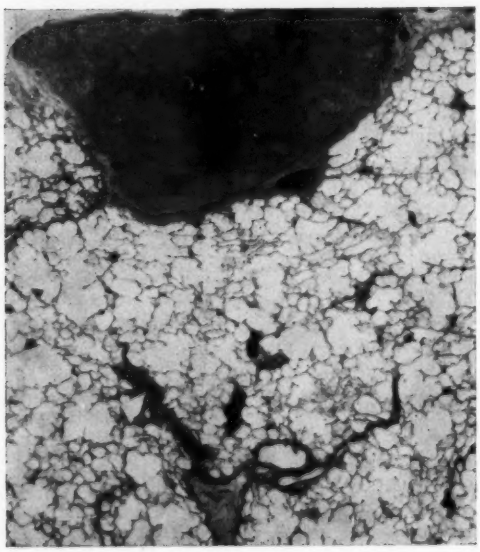

FIGURE XIVA.

Photomicrograph of a thick (200μ) section of a lung from an asthmatic patient, the bronchial arteries of which were injected with indian ink. The subpleural lymph node receives a rich bronchial arterial supply, one branch of which communicates through an anastomotic channel.

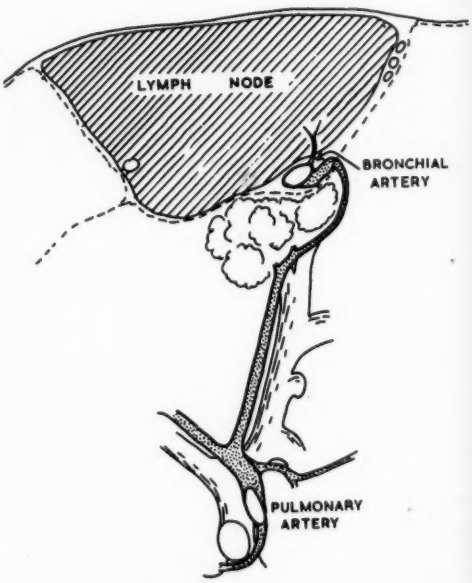

FIGURE XIVB.

Diagram to illustrate the feature shown in Figure XIVA. The pulmonary arterial branch supplies the underlying lung. The communication between the vessels is shown; in this case the pulmonary artery did not show evidence of sclerosis. (×12)

interarterial anastomic channels were few and small, but in all lungs, even in normal cases, the injection material eventually returned through the cut end of the main pulmonary artery (often before appearing in the pulmonary veins) and filled the pulmonary arterial tree. In emphysematous lungs the bronchial arteries were larger and more numerous. By following

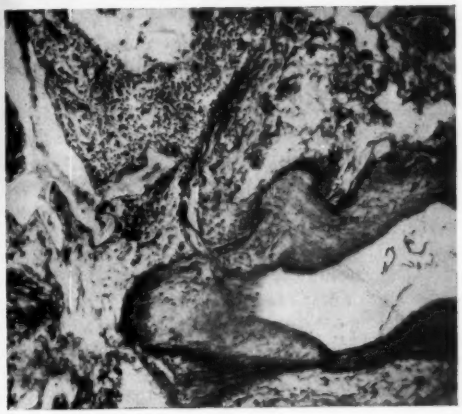

FIGURE XV

Photomicrograph showing, in an emphysematous lung, a pulmonary arterial branch with a considerably thickened intima, which is entered by a small bronchial vessel which, in adjacent sections, communicated with the lumen. The bronchial vessel lies in the wall of a heavily damaged bronchiole with evidence of acute bronchiolitis. (Elastin stain, ×60)

the stages of injection fluoroscopically it was evident that each enlarged branch eventually communicated with a pulmonary artery (Figure XII), the bronchial artery rarely filling with the radioopaque material beyond its anastomotic branch (or branches). In most cases the interarterial anastomotic channels were short and presumably connected a bronchial artery in the wall of a bronchus to the adjacent pulmonary artery. In some examples, however, a bronchial artery was not parabronchial throughout its entire course, but left the bronchus, traversed the lung and became subpleural (some bronchial arteries were subpleural in position from their origin in the mediastinum outwards, but such vessels were not demonstrated in normal lungs). All such arteries eventually communicated with a pulmonary vessel—usually an artery.

These subpleural bronchial arterial branches were commonly found in bullous emphysema, coursing over bullæ and entering subpleural scars (Figure XIII). This was seen well in apical lesions, particularly large scars of tuberculous origin, which received a rich bronchial arterial supply, both by parabronchial and by subpleural vessels; most of these could be demonstrated to anastomose with pulmonary arterial branches.

Similar extension of the bronchial blood supply with the formation of interarterial anastomoses was observed in bronchiectasis, the extent of the change being related to the severity of the bronchiectasis. Indeed, dilated bronchial arteries and anastomoses could be demonstrated by this method in any region in which there was macroscopic evidence of severe old inflammatory bronchial disease manifested by bronchiectasis, "scarring" or emphysema. The pattern was similar in all three, as well as in the two cases of mitral stenosis, in which there was insignificant chronic inflammatory disease but considerable pulmonary arterial sclerosis.

Histological examination of these anastomotic channels showed that they usually entered an

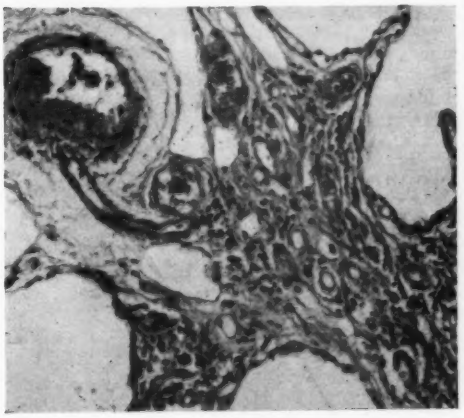

FIGURE XVI

Photomicrograph of a section from a bronchiectatic lobe. A small bronchial arteriole enters the thickened intima of a pulmonary arterial branch. The internal thickening is localized to one side of the lumen. The bronchial arteriole arises from a disorganized area of connective tissue containing islands of epithelium. Much bronchiolar obliteration has occurred in this region. (Hæmatoxylin and eosin, ×100)

obviously sclerosed pulmonary artery. Exceptions were found, one of which was a relatively wide vessel, there being a communication between a bronchial artery in a subpleural lymphatic nodule (in the lung of an asthmatic subject) and a pulmonary artery supplying the subjacent lung (Figure XIV). There was

little evidence of inflammatory damage to this lobule, and the artery was not sclerosed. Smaller interarterial anastomoses are common

FIGURE XVIIA.

Photomicrograph of a section of the wall of a small pulmonary artery adjacent to an ectatic bronchiole. A small vessel enters the lumen. (Hæmatoxylin and eosin)

in normal lungs in the region of the pleura (Küttner, 1878), and these appear to be enlargements of previously existing passages.

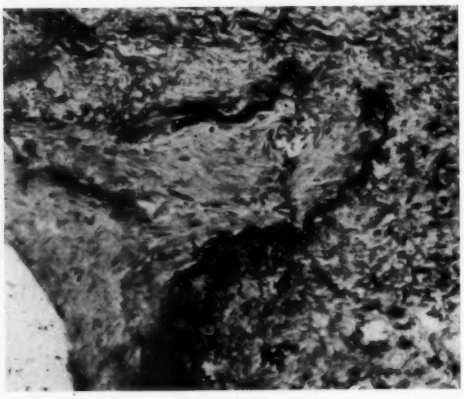

FIGURE XVIIB.

Same section as XVIIA. It is evident that this vessel traverses the almost occluded lumen of a branch of the remaining patent pulmonary arteriole. No muscle remains in the wall, and much of the elastic tissue is damaged. The small vessel was traced peripherally to communicate with bronchial arterial branches. (Elastin stain, ×90)

Interarterial communications leading to sclerosed pulmonary arteries were more common. Small communications of this type were often found in sections of areas in which there was evidence of considerable old damage to the related bronchi or bronchioles. They were found in both emphysema (Figure XV) and bronchiectasis (Figure XVI). In all these examples the sclerosis of the pulmonary artery was obvious, the anastomotic channel passing through a mass of thickened intima.

In larger arteries the interarterial anastomoses were often simply channels through the wall, but were occasionally much larger than capillaries; however, no example was found exceeding 100μ diameter. Tortuous anastomotic channels were also found, which at first sight appeared to be simply a bronchial arterial branch entering a sclerosed pulmonary artery. However, on closer examination, it was demonstrated that the small anastomotic vessels lay within the almost obliterated lumen of a branch of the pulmonary artery (Figure XVII). As this branch was traced peripherally, the remnants of the wall of this pulmonary arterial branch were eventually no longer discernable and the small patent vessel within the lumen led at length to a recognizable bronchial artery. Once the appreciation of this type of anastomosis was achieved, it led to the recognition of many obliterated pulmonary arteries that had been previously overlooked.

DISCUSSION

Many investigators have recorded observations on pulmonary arterial changes in emphysema—both on atheroma in the larger vessels and on sclerosis in the smaller. Waters (1862) found that, where there was significant pulmonary atheroma in emphysematous lungs, the aorta also was always grossly affected. Brenner (1935) demonstrated histological evidence of atheroma in 97% of lungs from adults regardless of the presence of pulmonary disease; he found that the severity of the atheroma was related to the age of the patient and the extent of the disease in the systemic arteries. A comparison of patients with and without emphysema enabled him to conclude that there was also some relation to the severity of the emphysema. Similar results were obtained in other chronic cardio-pulmonary diseases.

Likewise, sclerotic changes in the smaller arteries have been described in a high percentage of adults with no clinical cardio-pulmonary disease and with macroscopically normal lungs (Welch and Kinney, 1948; Civin and Edwards, 1951), as well as in most lungs showing evidence of cardio-pulmonary disease. It is generally agreed that the sclerosis is due largely to intimal thickening, and that there may or

may not be evidence of damage to the media. What has not been stressed is that there is convincing evidence that the intimal thickening in these vessels is in large measure (if not entirely) due to organization of thrombus, and that, in these small arteries, most such thrombi are due to inflammation adjacent to the artery and within its wall.

The evidence for this concept can be presented in two ways. The first group of observations indicates that the late stages of organization of thrombi are identical with what is described as "arterial sclerosis". The evolution of these changes has been briefly presented here. Many investigators have traced, usually in rabbits, the evolution of thrombi in the smaller pulmonary arteries produced by the injection of coagulants of different types into the systemic veins (Harrison, 1948, 1951; Wartmann *et alii*, 1951; Muirhead and Montgomery, 1951; McLetchie, 1952; Barnard, 1953, 1954). All these authors recorded changes similar to those described here in man. Harrison and Barnard stress strongly the close relation of changes produced in this way to those of naturally occurring arteriosclerosis.

Reports of similar stages of organization of thrombi in the pulmonary arteries of man are somewhat scattered. Recurrent pulmonary embolism either by thrombus (McKeown, 1952) or by tumour cells (Saphir, 1947) can lead to pulmonary arterial sclerosis. McKeown (1952) stated that these lesions are "almost identical with those found in pulmonary arteriosclerosis"; points of distinction were not made apparent. Similar lesions have also been reported in the rare condition of pulmonary arteritis and in *polyarteritis nodosa* (McKeown, 1952).

Far more commonly, fragments of the same series of changes in the organized thombus have been mentioned in numerous inflammatory conditions. Indeed it is so common that it rarely receives more than incidental acknowledgement, although the early stages of organization of such thrombosed vessels are commonly described in staphylococcal pneumonia. Different stages of organization of thrombi, which were undoubtedly due to associated respiratory tract infection (and in which, in several cases, staphylococci were cultured from the sputum), are illustrated by Frothingham (1929), Goedel (1930), Kershner and Adams (1948) and Ludwig (1951).

The second group of observations is that which demonstrates the close topographical relation of arterial sclerosis to lung tissue showing evidence of old inflammatory damage. Typical arteriosclerotic changes have been described in the small arteries in many specific and nonspecific lŏcalized inflammatory conditions. Particular note was taken of them by Cudkowicz and Armstrong (1952) in tuberculosis, and by Auerbach and Stemmerman (1944) in silicosis. In this study, regardless of the ætiology of old localized inflammatory lesions represented either by emphysema, "scarring" or bronchiectasis, some degree of sclerosis was found constantly in the damaged regions; it ranged from slight intimal thickening to complete occlusion. Few would doubt that this sclerosis was fundamentally inflammatory in origin.

It would be logically inconsistent to accept that the sclerosis of vessels in a localized area of old inflammation is due to organization of mural thrombi and to deny this explanation in more widespread or generalized inflammatory conditions. Generalized emphysema has been shown to be a result of damage to small air passages, the maximal injury occurring in the smallest bronchioles (McLean, 1957*b*, *c*). In the earliest stages of the condition, old damage was shown to be restricted largely to the terminal and respiratory bronchioles; as has been observed here, many of these lungs exhibit sclerosis of the arteries adjacent to these damaged passages with little change elsewhere. Moreover, in the same lungs, where the bronchiolar damage was greater so also was the sclerosis of the adjacent arteries.

These observations stress the inflammatory origin of arterial sclerosis in emphysema. What objections can be levelled at this conclusion? The main one is that sclerosis is common in "normal" lungs. The work of Neely, described by McKeown (1952), showed that after the third decade some sclerotic vessels can be demonstrated in all lungs. In a series of "normal" lungs from patients aged 50 to 70 years, 7% showed "marked sclerosis", similar changes being demonstrable in emphysema in 10% of cases without, and in 17% with, obvious increase in the weight of the right ventricle. These results demand comment. First, it is emphasized that the sclerosis cannot be assumed to be due to what has been termed "an aging process". It has been pointed out previously that histological evidence of diffuse old damage to bronchioles, with fibrous thickening and even occlusion, was regularly found in patients with macroscopically normal lungs in the age group studied by Neely (McLean, 1957*a*), so that sclerosis of the adjacent arteries may reasonably be regarded as inflammatory.

What is more surprising is the extraordinary distribution of "marked sclerosis" between

non-emphysematous and emphysematous lungs. It was observed in the present study, that, although sclerosis was found only in arteries adjacent to damaged bronchioles, in macroscopically normal lungs and in early generalized emphysema the extent of the sclerosis was poorly related to the extent of bronchiolar damage. This was not astonishing since, for instance, a severe staphylococcal infection may produce gross generalized arterial sclerosis without strikingly great evidence of bronchiolar damage (as in the case of Frothingham, 1929); on the other hand, repeated non-specific infections may, over the years, produce gross bronchiolar damage and emphysema without, during the less severe and more localized attacks of acute bronchiolitis, significant thrombosis in the wall of the adjacent arteries. The results obtained by Neely simply stress the readily verifiable observations that the extent of vascular thrombosis occurring during respiratory infections varies within wide limits.

The poor correlation between the vascular and parenchymal change in early generalized emphysema, however, must not be allowed to monopolize the whole prospect. This is the stage at which recognition of obliterated bronchioles is most difficult and where they have certainly been overlooked by most observers; in more advanced generalized disease and also in the localized lesions the close topographical relation of damaged and obliterated bronchioles to sclerosed arteries makes it clear that the changes formed in the earliest stages of emphysema form part of general pattern.

In this presentation the evolution of sclerosis of smaller pulmonary arteries from thrombi has been emphasized. That sclerosis can evolve in this way is regarded as proven. Whether sclerosis (including atherosclerosis) in the pulmonary or the systemic circulation can arise by any other means than by organization of thrombus is moot. Duguid (1946, 1948, 1949) is the main proponent of the concept that both atheroma and intimal sclerosis arise solely from mural thrombi. Whatever criticism may be levelled at this concept, it must be admitted that there is not, at present, a satisfactory alternative proposition. (This proposition is by no means exclusive of the influence of metabolic factors, particularly those related to cholesterol metabolism, on the development of atheroma; it is made clear in the review of Duff and McMillan (1951) that both local and general factors must be considered.)

The relation of pulmonary arterial sclerosis to increased functional resistance in the pulmonary circulation in emphysema is not clear. In cases in which the sclerosis is extreme and generalized, there is an undoubted increase in pulmonary vascular resistance. However, with lesser changes there is often no evidence of clinical disability, raised pulmonary arterial pressure or, at autopsy, right ventricular hypertrophy. This is evident from the above mentioned observations of Neely. McKeown (1952) concluded that pulmonary vascular sclerosis "cannot be regarded as a morphological basis for the (pulmonary) hypertension" in emphysema, but this conclusion is an oversimplification of an extremely complex situation.

It is necessary to point out that, as yet, the effect of structural changes in the genesis of pulmonary hypertension in emphysema has not been even approximately assessed. It is rendered particularly difficult by the lack of uniformity of vascular changes in the lung and the considerable functional reserve of the pulmonary vascular bed.

Structural changes may increase pulmonary vascular resistance at two main levels. The first is the reduction of the capillary bed that inevitably accompanies the disease. Alone, this probably is not of great significance, since many patients with gross emphysema do not have, at rest, an elevated pulmonary arterial pressure. However, in all cases this effect of the disease reduces considerably the functional reserve of the vascular bed.

The second is the question of sclerotic changes in the smaller arteries, which was considered by McKeown (1952). To assess the effects of these changes requires, above all, an accurate determination of the proportion of completely obliterated small arteries in damaged lungs. This obvious point has been omitted from consideration previously, presumably because the remnants of such vessels are frequently imperceptible. The only satisfactory alternative procedure would be to determine the number of remaining small arteries, an equally monumental task. Assuming either procedure to be practicable, it would then be necessary to compare lungs in which the arteries were fixed distended at a known pressure and by a standard technique, since intimal thickening not only decreases the lumen but also reduces the distensibility of the vessel—an effect of particular importance in the normally highly distensible small arteries of the lung. Ideally, it would be necessary to measure all the remaining arteries in the lungs, since it is clear that severe but patchy sclerosis would be of less functional significance than slight but generalized change. Finally, it should be pointed out that slight errors in measurement

would produce considerable errors since Poiseuille's Law, which states that the rate of (streamline) flow of fluid through a tube is proportional to the fourth power of the radius, applies approximately to the flow of blood through a vessel of arterial size.

However, impracticable though such an investigation might be with present techniques, it is abundantly clear that the measurement of a small number of arteries fixed at different degrees of distension in different lungs cannot be relied on to give more than a vague, and often misleading, indication of the effects of arterial sclerosis on vascular resistance, particularly as such measurements require the completely obliterated but inconspicuous small arteries which cannot be recognized easily, even with careful study of serial sections.

Superimposed on these as yet unassessed structural changes, important physiological mechanisms influence pulmonary vascular resistance. These mechanisms are undoubtedly of considerable significance in both normal and diseased lungs, but the precise manner in which they act is obscure. Certain stimuli are recognized to be associated with an increase in pulmonary vascular resistance; of these the most studied are anoxia (von Euler and Liljestrand, 1946; Motley *et alii*, 1947; Mounsey *et alii*, 1952; Yu *et alii*, 1953) and carbon dioxide retention (Harvey *et alii*, 1951; Yu *et alii*, 1953); yet opinion is by no means unanimous on the effects of either (Doyle *et alii*, 1952; Mounsey *et alii*, 1952). These stimuli are considered by some to act through the central nervous system (Halmágyi *et alii*, 1953; Stroud and Rahn, 1953); whereas others consider that the effect is local (Logaras, 1947; Liljestrand, 1948). Most observers regard the increased vascular resistance as being due to increased arterial tone, but evidence for the view that it may be greatly influenced by the tone of the bronchial musculature has been put forward by Rodbard (1953). Much clarification of the subject is obviously necessary.

It is these physiological mechanisms that have been invoked to explain those rare cases of primary pulmonary hypertension in which, at autopsy, considerable right ventricular hypertrophy is found but only slight changes have been demonstrated in the small pulmonary arteries. In most reports the histological study of the arteries was not extensive, and sometimes, even though many of the patients had given a history characteristic of "bronchiolitis obliterans" (McAdams, 1955), no evidence of inflammatory damage to bronchioles was mentioned—Case 3 of De Navasquez, Forbes and Holling (1940) can be cited as such an example; thus statements on the absence of vascular change are unconvincing. However, despite the morphological difficulties involved in demonstrating the absence of significant sclerosis (including complete obliteration) in these cases, there has undoubtedly been a number in which the sclerosis was slight in proportion to the hypertension. In some of these cases the hypertension was of considerable duration; this indicates that the sclerosis is not, as is sometimes inferred, simply a result of the hypertension, although (as in the systemic circulation) hypertension probably plays some role in the pathogenesis of the condition.

Sclerotic changes in the bronchial arteries show the same histological features as those in the pulmonary arteries. Significant sclerosis was seen most often in bronchiectasis, where the wall of the bronchus, in which the sclerosed arteries were found, showed much evidence of old inflammation. Bronchial damage of this degree was not common in emphysema; but when it did occur, the bronchus was ectatic, and, within the wall, sclerosed bronchial arteries were often observed. In arteries within more normal bronchi in cases of severe systemic hypertension only slight sclerosis was found. These observations further emphasize the importance of inflammation in producing vascular sclerosis in the lung.

The intima of sclerosed arteries—pulmonary as well as bronchial—often contained elastic fibres and also, occasionally, smooth muscle. New formation of elastic tissue has been discussed previously (McLean, 1957*a*); it has been shown that this occurs in any connective tissue subject to a repeated stretching force and so could be expected in an organized thrombus on the wall of an artery. The source of the muscle fibres is more debatable; it is likely that this is also new formation within the connective tissue given the appropriate, but at present unknown, stimulus. This is supported by the observations of Taylor (1954), who demonstrated new formation of smooth muscle in experimentally induced intimal thickening in the arteries of rabbits. Those not attracted by the general idea of metaplasia, such as Golowinski (1905), have suggested that muscle within the intima of similar arteries found elsewhere in the body grows out from the media. There is no direct evidence to support this suggestion; it is merely another conjecture which, furthermore, has less observational and experimental support than the hypothesis of metaplasia.

Von Hayek (1940*a* and *b*, 1952), Verloop (1948), and Lapp (1951) described, in "normal" lungs, occasional bronchial arteries with a thick well-formed musculo-elastic intima. Von Hayek and Lapp refer to these arteries as *Sperrarterien* (or shunt-arteries), since, in the material they examined, they found that these arteries emptied more or less directly into pulmonary vessels; both authors consider that these *Sperrarterien* have a special functional significance. Merkel (1942) found similar arteries without the anastomotic communications, and, from the observations presented here and from those of Liebow, Loring and Felton (1953), the same appearance is found in both pulmonary and bronchial arteries in damaged lungs. Liebow *et alii* therefore doubted that similar structures in normal lungs were of any special significance. In any case, the possibility that these *Sperrarterien* are also sclerosed vessels has not been disproven.

A small number of interarterial anastomoses of larger than capillary size is found in the normal lung. Tobin (1952) found such anastomoses in the newborn, and, from embryological studies, he concluded that these communications are "normal" structures. As Cudkowicz and Armstrong (1952) showed, these channels may be greatly enlarged in congenital heart disease. However, in chronic pulmonary disease the size and number of interarterial anastomoses are often very much greater than those in normal lungs. This has been demonstrated in tuberculosis (Cudkowicz, 1952), in bronchiectasis (Wood and Miller, 1938; Liebow, Hales and Lindskog, 1949) and in emphysema (Marchand, Gilroy and Wilson, 1950; Cudkowicz and Armstrong, 1953).

Cudkowicz (1952) concluded from his observations that these anastomoses are not simply enlarged existing channels, but that they formed by the recanalization of thrombosed pulmonary arteries by *vasa vasorum* (bronchial arterial branches), with the resulting formation of communications between the *vasa vasorum* and the new pulmonary arterial lumen at the site of thrombus. This conclusion is endorsed by observations made here, and also by those of Holman and Mathers (1929) and Johnson (1955), who found, some time after injecting emboli experimentally, that an enlarged bronchial artery led to the site at which the embolus had lodged in a small pulmonary artery.

As Wright (1938) pointed out, where pressure gradients are such that flow is maintained in a newly formed vessel, the vessel persists and enlarges. The stimulus which determines vessel growth is cellular activity in the tissue supplied. This is well shown in the embryo on the one hand and in the blood supply of tumours on the other. The activity of tissue and "demand" for nutriment in inflammatory states are commonly encountered phenomena. An everyday example of enlargement of minute vessels to a considerable size is the development of arteries in serosal adhesions or in the plastic surgeon's skin flaps. These anastomoses, originally of capillary size, like the subpleural bronchial arterioles and capillaries in normal lung, may therefore, under appropriate conditions, enlarge and subsequently even develop a musculo-elastic wall. This is the best and most practicable explanation for the relatively large subpleural bronchial arteries observed in emphysema and is adequate to explain the musculo-elastic wall of the larger interarterial anastomotic channels.

The functional significance of these anastomoses may not be great. Blood flow through the anastomotic vessels from the bronchial arteries to the pulmonary arteries might slightly increase the work of the left side of the heart and produce focal hypertensive regions in the pulmonary circulation. During cardiac catheterization "pulmonary capillary pressure" is sometimes excessively high and arterial blood is withdrawn; presumably in these cases the catheter occludes a pulmonary artery proximal to an interarterial anastomosis. Similarly in angiocardiography, the pulmonary arteries fill poorly with radioopaque material in diseased areas such as tuberculous lesions (Carvalho, 1950), although, in such, patent pulmonary arteries can be demonstrated histologically (Cudkowicz, 1952). One functional implication, therefore, is that deoxygenated blood is shunted away from damaged parts of the lung. In generalized emphysema these anastomoses are not often strikingly numerous or large, and it is unlikely that, in most cases at least, they could produce *per se* a significantly increased pulmonary vascular resistance.

Consideration of all the vascular changes that are found in emphysema leads to the conclusion that there is no change specific to the condition; similar vascular lesions are found in all chronic inflammatory disease of the lung as well as in other conditions. These changes in chronic pulmonary inflammations are identical in localized and generalized conditions, of both specific and non-specific ætiology, regardless of whether they are manifested primarily by emphysema, "scarring" or bronchiectasis. Thus there is no evidence that emphysema results from vascular lesions.

At the same time we should not overlook the occlusion of many vessels, which are possibly

more numerous than is at present realized, because like obliterated bronchioles they are difficult to recognize. These may play some part in the full development of the condition, which is primarily due to mechanical factors. To what extent such disturbance of the blood supply will affect the nutrition of alveolar walls is difficult to assess, but, until some method of providing more direct evidence is obtained, it seems probable that they may play some, even though small, part in the loss of elasticity and atrophy of alveolar walls which are subjected to increased pressure. Also producing this change, and probably of greater significance, is the direct effect of persistently raised intraalveolar pressure during the disruptive phase on the blood flow through capillary mesh of the alveolar walls. However, since specific vascular changes are, to a great extent, unrelated to the presence or absence of emphysema it is clear that no vascular change occupies a significant primary ætiological rôle.

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Proceedings of The Royal Australasian College of Physicians

ORDINARY MEETING, 1957

An ordinary meeting of The Royal Australasian College of Physicians was held in Sydney from October 15 to 19, 1957. The President, Dr. E. G. Sayers, was in the chair.

Scientific Sessions

Two scientific sessions were held in the Stawell Hall of The Royal Australasian College of Physicians. The following contributions were given: "The Increasing Incidence of Varicella Pneumonitis in Adults", by H. McLorinan; "The Presence of Circulating Complement-Fixing Antibodies to Human Tissue Antigens as Evidence for Auto-Immune Mechanisms in Disease", by I. R. Mackay; "The Mechanism of Anæmia in Leukæmia", by Ivan S. Epstein; "Preliminary Observations on the Significance of Tissue Arsenic Levels in Cases of Acute and Chronic Arsenical Poisoning", by H. Lander; "Prognostic Factors in the Treatment of Myocardial Infarction", by M. K. Gray; "Mitral Stenosis as it Presents in General Medicine", by James Isbister; "Aortic Valvotomy for Aortic Stenosis", by G. V. Hall; "Hæmodynamic Studies in the Assessment of Aortic Stenosis", by John B. Hickie.

The following summaries have been received from individual contributors.

H. McLorinan, in his paper on "The Increasing Incidence of Varicella Pneumonitis in Adults", stated that during the last six years, 12 cases of primary varicella pneumonia had been admitted to Fairfield Hospital, Melbourne, with two deaths. All the patients had been male adults. Irritating cough with dyspnœa and cyanosis in the severe cases were the salient clinical features. An oxygen tent was considered to be life-saving in these patients, and it was recommended that it should be used as soon as possible. The X-ray findings in the chest were typical and were described as "widespread nodular densities superimposed on markedly increased broncho-vascular markings". The most striking feature of the autopsy findings in the two fatal cases had been the presence of varicella lesions on the visceral pleura. The lungs had shown evidence of œdema and congestion and areas of hæmorrhage. There appeared to be no satisfactory explanation for the increase in severity of chickenpox in adults, although similar happenings had been noted in relation to poliomyelitis and infectious hepatitis, particularly during the last decade.

Ivan S. Epstein discussed "The Mechanism of Anæmia in Leukæmia". It was stated that the mechanism of anæmia in leukæmia had been investigated by utilizing radioactive iron (^{59}Fe) to assess erythropoiesis and radioactive chromium (^{51}Cr) to assess red cell survival in untreated cases of leukæmia. Of four patients with acute leukæmia, two had uncompensated hæmolysis, one had partially compensated hæmolysis, and one had production failure but normal survival. The seven chronic lymphatic leukæmia patients could be divided into three groups, namely, (i) the group with normal red cell production and survival, (ii) the hæmolytic group, and (iii) the aplastic group. The chronic myeloid leukæmia patients had also shown varying combinations of production failure and diminished survival; but they had all exhibited an unusual ^{59}Fe uptake pattern suggesting the possibility of active extramedullary erythropoiesis in this disease. The abnormal mechanisms had been reversed when hæmatological remission was induced.

M. K. Gray presented his paper on "Prognostic Factors in the Treatment of Myocardial Infarction", in which it was reported that, following the work of Russek and others, a study had been commenced about four years previously on the grading of myocardial infarction and the assessment of important factors in prognosis. The results in 150 cases were presented. The criteria for the major type of infarction had been (i) shock, (ii) heart failure, (iii) diabetes, (iv) the second or further attack, (v) an electrocardiogram with prominent and wide *Q* in significant leads. The following figures had been supplied: Death rate: total, 25/150 (15%); major, 24/119 (20%); minor, 1/31 (3%). Analysis of major deaths: shock, 10/16 (62·5%); heart failure, 9/10 (90%); diabetes, 3/9 (33%); second or further attack, 4/26 (16%); electrocardiogram alone, 1/61 (1·6%). It was therefore obvious that the electrocardiogram alone was no guide. Transferring the 61 in that group to the "minor" group gave the following corrected death rate: total, 25/150 (17%); major, 23/58 (38%); minor, 2/92 (2%). The use of anticoagulants in this "minor" group with such a low death rate was considered unnecessary.

James Isbister, in his paper on "Mitral Stenosis as it Presents in General Medicine", reported that a group of patients admitted into general medical wards between January, 1950, and September, 1957, at Prince Henry Hospital, Sydney, had been studied. Approximately 100,000 patients in all had been seen, and of those 115 had been personally examined and diagnosed as having mitral stenosis. Readmissions had been rejected. All patients had been admitted to hospital on account of acute illness, and consequently the relatively asymptomatic ones and antenatal cases had not been included; in other respects the cases had come direct from general practice and had not been selected. This group of patients had been different from that seen in special cardiac clinics. The purpose of the paper was to present (i) an analysis of the diagnostic efficiency of referring general practitioners and of the admitting resident medical officer and (ii) an analysis of the clinical characteristics of the group. The referring

general practitioner had diagnosed 10% as having mitral stenosis, the resident medical officer had diagnosed 44%, and it appeared that 30% had been previously diagnosed. Diagnostic efficiency had appeared to fall with increasing age and the presence of auricular fibrillation. A knowledge of a previous history of rheumatic fever had doubled the diagnostic efficiency of the resident medical officer. The following clinical features had been analysed: congestive cardiac failure, pulmonary congestion, cerebral embolism, anginal pain, subacute bacterial endocarditis, associated valvular disease and deaths. Compared with that of other groups, the average age had been higher, auricular fibrillation more common, and cerebral embolism much more common. Although only a few of the group had been referred for consideration of surgery, it had appeared that a large number of cases of mitral stenosis in the community were not being diagnosed. The efficiency of diagnosis should be improved not only for present surgical indications, but also in case suitable treatment of mitral incompetence became available. The problem appeared to be one of initial diagnosis by doctors as a whole, rather than assessment by the cardiologists.

CLINICAL MEETINGS

Two clinical meetings were held at St. Vincent's Hospital, Darlinghurst, and at Sydney Hospital, Macquarie Street, Sydney. The following contributions were given at St. Vincent's Hospital: "Wegener's Granuloma", by Peter Harvey; "Sarcoidosis with Neurological Complications", by J. Hassall; "Phæochromocytoma of Long Duration", by J. Kempson Maddox; "Argentaffinoma Syndrome of Pulmonary Origin", by A. Freedman. The following contributions were given at Sydney Hospital: "The Artificial Kidney in Sydney Hospital", by H. M. Whyte; "Peutz-Jegher's Syndrome", by D. J. Harbison; "Agammaglobulinæmia", by F. Hales Wilson; "Periodic Paralysis and Nephropathy due to Hypokalæmia", by K. D. G. Edwards (introduced by G. E. Bauer).

ANNIE B. CUNNING LECTURE ON NUTRITION

The tenth Annie B. Cunning Lecture on Nutrition was delivered in the Winthrop Hall of the University of Western Australia on the evening of Thursday, August 8, 1957. The lecture was delivered by Dr. H. W. Bennets, D.V.Sc., Principal of the Animal Health and Nutrition Laboratory of the Department of Agriculture of Western Australia, on the subject "A Nutritional Dilemma". The lecture was attended by a distinguished audience of 500 persons, and guests were later entertained at supper in the Refectory.

MEMBERSHIP

Admission of Members. The following candidates who were successful at an examination held in Australia in August-October, 1957, were admitted to Membership on October 15, 1957: H. J. H. Colebatch and A. M. Rankin, of Western Australia; P. R. Hodge and S. Posen, of South Australia; G. W. Crock, D. J. Fone, N. B. Pinkus, B. A. Smithurst and G. R. Wagner, of Victoria; J. C. H. Morris of Tasmania; W. G. Grigor, D. A. Handley, B. R. M. Hurt, J. McRae and R. A. Mellick, of New South Wales; B. T. Emmerson, G. A. Hocker and K. J. Murphy, of Queensland; and under Article 37, R. W. Hawker of Queensland.

Obituary. Council records with regret the death of Dr. Mervyn Archdall, of New South Wales, Dr. Walter Gilmour, of New Zealand, Dr. Valentine McDowall, of Queensland, and Dr. E. S. Morris, of New South Wales, who were Fellows of the College.

Membership Roll. The College now has a roll of 324 Fellows and 520 Members.

GENERAL

Election of Councillors. Dr. A. W. Morrow, of New South Wales, was elected by ballot of the Fellows to a vacancy on the Council, under Article 9*a*, and Dr. E. H. Roche, of New Zealand, by ballot of the New Zealand Fellows, to a vacancy under Article 9*aa*.

Scholarships. The Wunderly Travelling Scholarship in Thoracic Diseases for 1958 was awarded to Dr. John Read, of New South Wales. The Travelling Scholarship in Medicine or the Allied Sciences for 1958 was awarded to Dr. H. Lander, of South Australia.

Research Grants. The following grants were awarded from the Adolph Basser Research Fund of the College: (*a*) A fellowship for a period of five years to Dr. R. A. Joske, of Melbourne, for research into gastro-intestinal disease and chronic liver disease, in the Department of Medicine at the University of Western Australia. (*b*) A Fellowship for a period of five years to Dr. Denis F. J. Halmagyi, formerly of Hungary, for research into the pulmonary circulation in the Department of Medicine at the University of Sydney. (*c*) A fellowship for one year to Dr. K. D. G. Edwards, of Sydney, for research into body fluid, electrolyte and renal function, in the Clinical Research Department of Sydney Hospital. (*d*) A grant-in-aid to Dr. B. P. Billington, of Sydney, for part-time research into blood groups and disease, in the Department of Medicine at the University of Sydney.

Therapeutics Assessment Committee. A Therapeutics Assessment Committee has been appointed, consisting

of Professor C. R. B. Blackburn, Professor R. H. Lovell, Dr. H. O. Lancaster, Dr. Keith Fairley, Professor J. G. Hayden (Chairman) and Dr. M. V. Clarke (Secretary). The Committee will hold office until the Annual Meeting in 1960.

Representative of the College. Professor J. G. Hayden has been reappointed as College representative on the National Health and Medical Research Council of Australia.

Appointment of Examiners for Ryan Scholarships, 1958. Council has appointed Dr. R. M. Biggins, of St. Vincent's Hospital, Melbourne, and Dr. Margaret Henderson, of the Royal Melbourne Hospital, Parkville, as examiners for the Margaret and T. F. Ryan Scholarships in 1958.

Purchase of Harvey Film. The film "William Harvey and the Circulation of the Blood" has been purchased by the College from the bequest of the late Dr. Robert Scot Skirving, and it is intended that this film be made available widely for screening to medical audiences throughout the Commonwealth.

Proposed Visit of Professor Saul Adler. It is hoped that Professor Saul Adler, of Israel, will visit various States of the Commonwealth in 1958 under the auspices of this College.

Gifts to Library. Gifts to the Library have been received with appreciation from the Royal College of Physicians of London, the Royal College of Physicians of Edinburgh, Professor W. S. Dawson, Dr. K. B. Noad and Dr. B. P. Billington.

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